THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XXXI MAY-JUNE, 1955 NUMBER 3

FAT EMBOLI IN GLOMERULAR CAPILLARIES OF CHOLINE-DEFICIENT RATS AND OF PATIENTS WITH DIABETIC GLOMERULOSCLEROSIS *

W. STANLEY HARTROFT, M.D.†

From the Banting and Best Department of Medical Research and the Department of Pathology, University of Toronto, Toronto 5, Ont.

In their original description of the renal lesions that are now known by their names, Kimmelstiel and Wilson[1] recorded the presence of fat in some of the affected glomeruli. At least seven subsequently published reports of this condition have described glomerular lipid deposits.[2-8] Wilens, Elster, and Baker,[8] in a detailed study, compared the frequency with which glomerular lipidosis occurred in Kimmelstiel-Wilson lesions with its incidence in a number of related conditions, including chronic glomerulonephritis, arteriolar nephrosclerosis, and amyloidosis. Only in intercapillary glomerulosclerosis did they find the quantity of glomerular fat directly proportional to the severity of the lesions. These authors found that fat deposits in Kimmelstiel-Wilson lesions were localized to either the capillary tuft (presumably the wall) or the intercapillary space. Evidence will be presented here that in 75 per cent of a small series of lesions, examined in frozen sections, glomerular fat could be found in embolic form *within* the glomerular capillary lumina.

The search for embolic fat in glomeruli of diabetic patients with Kimmelstiel-Wilson lesions was initiated after it had been observed that fat emboli may lodge in glomerular capillaries of choline-deficient rats and produce lesions that closely resemble intercapillary glomerular sclerosis in diabetic man. The hepatic origin of fat emboli in the experimental animals had been demonstrated earlier by my associates

* Supported in part by grants from the National Research Council of Canada and the Nutrition Foundation, Inc.

Presented in part at the Fiftieth Annual Meeting of the American Association of Pathologists and Bacteriologists, St. Louis, April 2, 1953.

Received for publication, September 9, 1954.

† Now at the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.

and me.[9,10] But when those publications were written, the effects of glomerular fat emboli had not progressed to the eventual formation of lesions of the type described by Kimmelstiel and Wilson in man. The findings in the animals will be presented first, followed by results of the survey of frozen sections of diabetic humans and of a number of cases that served as controls.

Materials and Methods

Animal Material

Observations on the rat are based on the examination of over 150 male Wistar rats, initially weighing between 180 and 200 gm., that had been fed a choline-deficient diet* for periods ranging from 4 months to 1½ years. Fifty comparable animals were fed the same basal diet supplemented with choline chloride (0.35 per cent), and 20 others which were fed a stock diet (Purina Chow) ad libitum served as controls. Animals were killed at intervals during the course of the experiment by ether inhalation in a closed chamber, either for study of the development of hepatic, renal, and other lesions, or because they were moribund. At necropsy it was found that most of the choline-deficient rats had developed hepatic cirrhosis and lesions of the cardiovascular-renal system of the types we have reported elsewhere.[9-12] Only findings considered pertinent to the subject of this paper will be reported here.

At necropsy, blocks of renal tissue were fixed by immersion in formol-calcium solution[13] or in Bouin's solution. After 12 to 18 hours in the latter, the blocks were dehydrated and cleared in multiple changes of absolute isopropyl alcohol using an Autotechnicon. Infiltration with paraffin was carried out *in vacuo* and sections were cut at 5 μ and routinely stained with hematoxylin and eosin. Other sections were stained by a variety of methods including the periodic acid-Schiff's (PAS) technique of McManus,[14] demonstration of reticulin by silver precipitation,[15] ceroid,[16] hemosiderin,[17] and basement membranes.[18] The formalin-fixed blocks were frozen and cut at 5 μ. They were stained to demonstrate deposits of fat by Wilson's modification[19] of Lillie's supersaturated isopropanol technique.[16]

* The hypolipotropic diet was of the following percentage composition: peanut meal (solvent process), 6; alpha soya protein (Glidden's), 6; hydrogenated vegetable fat (Primex), 20; salts, 3; vitamin mixture, 1; Cellu flour, 2; sucrose, 61.8; cystine, 0.15; alpha-tocopherol acetate, 0.035; and cod liver oil concentrate, 0.015. The preparation of the diets, ingredients of the salt mixture, and of the vitamin mixture, and details concerning the care of the animals have been published previously.[10]

Human Tissues

Frozen sections were cut from formalin-fixed renal tissue obtained at necropsy from 16 diabetic patients with Kimmelstiel-Wilson's lesions, from 22 diabetic patients in whom this type of lesion had not been demonstrated, from 20 cases of alcoholic cirrhosis, and from 10 patients with a variety of miscellaneous lesions including acute, subacute, and chronic glomerulonephritis, myxedema (2 cases), and xanthomatous biliary cirrhosis (one case). These frozen sections were stained for fat by the same method employed for the animal material. A variety of special stains were carried out also on paraffin sections of the human kidneys.

Results

Animal Material

Fat emboli were conspicuous and easily demonstrable not only in kidneys of the choline-deficient rats, but also in lungs and hearts, as we have previously reported.[10] Emboli were found in arcuate arteries, interlobular arterioles, and other vessels, and most frequently in the capillaries of the glomerular tufts (Fig. 1). Only rarely had fat emboli lodged within *all* the capillaries of any one glomerulus; far more frequently the distribution of the lipid was focal, being confined to but one tuft. In the region of the emboli, capillaries were distended with red blood cells, presenting a picture of stasis. Ink injections, as previously reported,[10] demonstrated that in glomeruli of this type the fat emboli effectively obstruct inflow of the injection media. This finding suggests that capillary stasis and dilation is the result of obstruction of blood flow by the fat emboli (Fig. 2).

In addition to congestion and dilation, in paraffin sections of the kidneys degenerative changes in the walls of the glomerular capillaries were demonstrated. Thickening of basement membranes in these regions was encountered commonly. In some glomeruli, considered to represent early stages of the lesions, homogeneous eosinophilic material was encountered, which suggested exuded plasma in and between glomerular capillary walls (Fig. 3).

The late stages of the glomerular changes in the rat closely resembled Kimmelstiel-Wilson lesions of the types described as focal and diffuse (Figs. 4 and 8). As illustrated, the focal lesions achieved relatively great dimensions, taking up considerable portions of the glomeruli. At this stage, it became difficult to localize the lesions precisely, as entire portions of glomerular capillary tufts were replaced by relatively homogeneous, eosinophilic material (Figs. 6 and 7). This

material was strongly positive to PAS reagents. It also stained brown-black by silver methods. Histochemical tests for ceroid and hemosiderin were negative, but in frozen sections stainable lipid was always found and occasionally cholesterol deposits could be demonstrated. The latter were never observed in early stages of the lesions.

The incidence of the lesions varied with the length of the time the animals were fed the hypolipotropic diets and with the sensitivity of the individual rat to choline deficiency, as judged by the severity of the hepatic lesions. Rats in which advanced stages of grossly nodular cirrhosis were present invariably showed numerous glomeruli in all stages of sclerosis. These animals exhibited other extra-renal lesions, including myocardial fibrosis, aortic sclerosis, lipidosis and hyperplasia of coronary arteries, ceroid and fat deposits in lymph nodes, and testicular atrophy. The retinal vessels of these animals have not yet been examined.

In summary, the glomerular lesions in choline-deficient rats were initiated by vascular plugging of glomerular tufts by embolic fat. Dilation and stasis of capillaries in which lipid obstructed the lumina was followed by focal degenerative changes usually restricted to one tuft or lobule of the glomerulus. Eosinophilic coagula appeared in and between walls of dilated capillaries. Eventually, considerable portions of the glomerular tuft were replaced by relatively homogeneous material that stained positively with PAS and silver stains. Less frequently, some glomeruli became diffusely involved, with an end result that resembled the diffuse type of Kimmelstiel-Wilson lesion of man. Lesions of either focal or diffuse type were most severe and frequent in animals in which the other stigmas of choline deficiency were most developed.

Human Material

Because the glomerular lesions in the choline-deficient rats appeared to resemble so closely Kimmelstiel-Wilson changes in diabetic man, and were initiated by fat emboli that plugged glomerular capillaries, a search was made for similar emboli in frozen sections of kidneys obtained at necropsy from diabetic and other patients. The numbers and types of cases studied have already been outlined.

The results can be summarized briefly as follows. In the kidneys of 16 diabetic patients in which Kimmelstiel-Wilson lesions were present, intraluminal fat could be clearly demonstrated within glomerular capillaries in 12 (Figs. 9 and 10). In these instances, fat could be demonstrated also within other vessels, both preglomerular and post-

glomerular (Fig. 11). In frozen sections of kidneys from only 3 of 22 diabetic patients in which Kimmelstiel-Wilson lesions had not been demonstrated, fat emboli were present in glomerular capillaries. Of 20 cases of alcoholic cirrhosis, fat was found within glomerular vessels in 4. In kidneys from 10 patients with miscellaneous lesions including various stages of glomerulonephritis, 2 cases of myxedema (treated) and one case of xanthomatous biliary cirrhosis, fat frequently was found deposited in various portions of arteriolar and glomerular capillary walls (as reported by Wilens *et al.*[8]), but intravascular fat could not be demonstrated convincingly in any.

Although fat was undoubtedly located ***within*** the lumina of the glomerular capillaries in the 12 patients with Kimmelstiel-Wilson lesions, more was present in the walls of the vessels, between them, in the space of Bowman, in the glomerular capsule, and even in the basement membranes of the tubules. We have also observed lipid in all these regions in a case of spontaneous diabetes in a dog in which Kimmelstiel-Wilson lesions were demonstrated.[20]

Intravascular fat in the diabetic patients was present in the form of solid plugs, and was intensely sudanophilic. In some instances, the plugs presented a crystalline appearance, but more frequently they were homogeneous. Kidneys that contained fat emboli exhibiting crystalline structure were carefully searched for deposits of ceroid, because the appearance of the sudanophilic material suggested the presence of this pigment. In the cases examined to date, ceroid could not be demonstrated. Some of the glomerular capillaries containing lipid were enormously dilated and in these vessels red blood cells frequently were intermingled with the fat. The appearance and distribution of fat in positions within the glomerulus other than intravascular will not be described, as my observations on this point are in agreement with the findings of others.

Fat in the renal vessels of the 4 alcoholic patients was in small droplet form; in some instances it was almost granular, and the intensity of the sudanophilia was not as great as in the diabetic patients. None of the alcoholic patients exhibited glomerular capillary dilation and in none could changes be found that resembled Kimmelstiel-Wilson lesions at any stage in their development.

In a few instances, intravascular fat in the diabetic patient was associated with exudation of plasma into vessel walls (Fig. 7), presenting an appearance similar to the early stages of the lesions in choline-deficient rats. Emphasis is given this point because plasma exudates associated with obstruction of the glomerular capillary lumen may be

an important factor in the pathogenesis of the lesions in both man and rat.

Sections of the livers of the diabetic patients with Kimmelstiel-Wilson lesions were searched for fatty cysts of the type I have described previously in both the choline-deficient rat[21] and alcoholic man.[22] Although cysts were found in some instances, I was unable to demonstrate any correlation between the presence of fatty hepatic cysts, the incidence of intravascular renal fat emboli, and the occurrence of glomerular lesions.

Discussion

Pathogenesis

Since their discovery nearly 2 decades ago, Kimmelstiel-Wilson lesions have been the subject of much speculation regarding their pathogenesis and nature. Evidence presented here concerning the human lesions cannot do more than suggest certain hypotheses; but in the animals it has been possible to follow every stage in the development of nodules that in many respects simulate those in man. Because the sequence of events in choline-deficient rats suggests certain pathways for further investigation of the diabetic changes, the pathogenesis of the animal lesions will be discussed.

Arrival of fat emboli in the capillary glomerular tufts in which they become entrapped is clearly the initial event in the development of nodular glomerular sclerosis in the choline-deficient rat. Emboli have been observed repeatedly in glomeruli that are otherwise relatively normal, so that the time relationships in this regard appear clear; but only rarely have glomerular lesions been encountered in which fat has not been present (usually within capillary lumina, although sometimes in or around walls of vascular lumina). Subsequent events in the rats indicate that obstruction of blood flow in capillaries plugged by fat is associated with their dilation, stasis, and plasma-exudation. Mixtures of blood and fat in the distended capillary lumina are eventually replaced by relatively structureless PAS-positive and eosinophilic bodies. The picture at this stage (Figs. 6 and 7) closely resembles Kimmelstiel-Wilson lesions of the focal type found in diabetic man. The unique character that these spheroid bodies assume in the animals has not been entirely explained, but it is possible that the special characteristics by which the glomerular filter differs from other capillaries in the body may be largely responsible for the phenomenon. That blood passing through glomerular capillaries loses approximately 20 per cent of its fluid content, not only may play a rôle in the development of the lesions but also may be responsible for

emboli becoming entrapped more frequently at these sites than appears to be the case for capillaries elsewhere in the body, with the possible exception of the lungs.[10]

Demonstration of fat emboli in glomeruli of diabetic man suggests, of course, that here, too, the sequence of events may be initiated by lipid plugs in capillaries. I am unaware of any previously published evidence to suggest that renal fat emboli in diabetic patients may play this rôle. Anderson[23] suggested that slowing or cessation of capillary blood flow may be responsible for development of glomerular sclerosis because examination of serial sections indicated that capillary obstruction induced by fibrin thrombi initiated the events. He demonstrated lipid material in the lesions but did not suggest that it was responsible for capillary obstruction, pointing out that the shape of the focal lesions in the glomeruli is exactly the same as that of capillary bulging in surgical fat embolism. Possibly Anderson's theory accounts for initiation of the sequence of events in some instances of glomerulosclerosis, but the almost constant presence of lipid deposits in some portion of the lesions must now be accepted. The frequent demonstration of actual intraluminal fat emboli in early stages of the human lesions suggests strongly that, as in the rat, fat emboli may frequently, if not always, initiate capillary obstruction. If fat emboli do play such a rôle, some other factor or factors, such as elevation of blood pressure, are probably necessary for the subsequent course of events that leads to formation of nodules, else one might expect to find them in alcoholic patients and others with similar disturbances of fat metabolism.* Wilens *et al.*[8] emphasized the importance of lipid deposits in glomerulosclerosis, and although they did not describe actual fat emboli in affected glomeruli, these authors felt that hyperlipemia in the early stages of hypertension was an important cause before sufficient elongation and narrowing of glomerular capillaries had developed to prevent elevation of intraglomerular pressure.

Source of Fat Emboli

In choline-deficient rats, there is little doubt that renal fat emboli come from the liver. Ruptured fatty cysts[9] have been demonstrated repeatedly to liberate lipids into torn hepatic sinusoids.[10] Frequently, ceroid pigment is deposited in large amounts in livers of choline-deficient rats, and ceroid emboli have been encountered in lungs and

* Lesions in glomeruli of 19 of 25 patients with "uncomplicated cirrhosis" appeared to resemble intercapillary glomerulosclerosis of Kimmelstiel-Wilson, in a series reported by Baxter and Ashworth.[24]

kidneys of these animals. These emboli probably originated from the liver, because primary deposition of this pigment has not been demonstrated in any other organ. If the evidence indicates that ceroid emboli in the kidneys originate from the liver, it would seem reasonable to assume that the fat emboli do also.

Source of the renal fat emboli in diabetic man is not so obvious. I have not been able to demonstrate any correlation between the incidence of glomerular fat emboli and fatty cysts in the livers of diabetic patients at necropsy. It is possible, of course, that cysts may have been present in these patients sometime before death, and that examination of their livers at earlier stages in the disease would have yielded additional information. Liver biopsy in diabetic patients in whom active progression of glomerular lesions has been diagnosed before death might be rewarding. Only recently it has been reported that fat emboli are encountered frequently at necropsy in the lungs of diabetic patients.[24]

Hyperlipemia is a well recognized and commonly accepted feature of diabetes. Loss of 20 per cent of the plasma from glomerular blood could precipitate lipid in hyperlipemic plasma as fatty plugs within the capillaries, particularly if intraglomerular pressure were elevated as suggested by Wilens.[8] In this event, it might not be correct to call the plugs emboli, and perhaps the fatty material should be regarded instead as lipidic thrombi. Further investigation is necessary before the exact nature and origin of the intraluminal lipid in diabetic man will be understood.

Although choline deficiency is responsible for the development of the lesions in rats that simulate glomerulosclerosis in man, there is no evidence to suggest that hypolipotropic states are involved in Kimmelstiel-Wilson disease. Disturbances of fat metabolism and transport may be the factor common to both choline-deficient rats and diabetic man. This possibility is supported by the fact that not only choline-deficient rats and untreated diabetic humans develop fatty livers, but also ocular lesions,[25] aortic sclerosis, and coronary arterial lipoidosis[12] are encountered in both states. Some of these lesions in the rat differ in details from their counterparts in diabetic man, but species-differences may account for discrepancies. Thus, the explanation for the development of somewhat similar pathologic changes in kidneys, hearts, and vessels of the choline-deficient rat and diabetic man may lie in a fundamental disorder in metabolism and transport of fat in both instances.

Summary

Multiple fat emboli have been encountered in the glomerular capillaries of choline-deficient rats. Lesions induced by the emboli eventually simulate both focal and diffuse types of the Kimmelstiel-Wilson lesions in diabetic man.

Fat in the lumina of glomerular capillaries was found in frozen sections of kidneys of 75 per cent of diabetic patients with Kimmelstiel-Wilson disease and of 15 per cent without glomerular sclerosis. Vascular lipid not associated with glomerular disease was found in a small percentage of cases of alcoholic cirrhosis, but was absent in a variety of miscellaneous forms of renal disease unassociated with diabetes.

Glomerular sclerosis in choline-deficient rats develops from embolic plugging of glomerular capillaries by fat. The stages in the course of the development of the lesions are described; they indicate their obstructive nature. The liver is probably the source of the fat emboli in the rats.

The source of fat plugs in glomerular capillary lumina of human diabetic patients is not obvious but may be associated with the existence of hyperlipemic states in the presence of elevated intraglomerular pressure. Evidence obtained from the present investigation and from those previously reported in the literature strongly suggests that glomerulosclerosis may result from capillary obstruction. It is possible that plugging of glomerular capillaries by fat either entrapped as emboli or precipitated *in situ* initiates the series of events that lead to formation of Kimmelstiel-Wilson lesions in diabetic man.

I am grateful to both Professor C. H. Best in the Banting and Best Department of Medical Research and Professor John Hamilton in the Department of Pathology, University of Toronto, for their generous assistance and helpful advice during the course of this investigation.

REFERENCES

1. Kimmelstiel, P., and Wilson, C. Intercapillary lesions in the glomeruli of the kidney. *Am. J. Path.*, 1936, **12**, 83–97.
2. Anson, L. J. Intercapillary glomerulosclerosis. *South. M. J.*, 1938, **31**, 1272–1275.
3. Newburger, R. A., and Peters, J. P. Intercapillary glomerulosclerosis. A syndrome of diabetes, hypertension and albuminuria. *Arch. Int. Med.*, 1939, **64**, 1252–1264.
4. Derow, H. A.; Altschule, M. D., and Schlesinger, M. J. The syndrome of diabetes mellitus, hypertension and nephrosis. A clinical and pathological study of a case. *New England J. Med.*, 1939, **221**, 1012–1015.

5. Allen, A. C. So-called intercapillary glomerulosclerosis—a lesion associated with diabetes mellitus. Morphogenesis and significance. *Arch. Path.*, 1941, **32**, 33–51.

6. Porter, W. B., and Walker, H. The clinical syndrome associated with intercapillary glomerulosclerosis. *J. A. M. A.*, 1941, **116**, 459–464.

7. Laipply, T. C.; Eitzen, O., and Dutra, F. R. Intercapillary glomerulosclerosis. *Arch. Int. Med.*, 1944, **74**, 354–364.

8. Wilens, S. L.; Elster, S. K., and Baker, J. P. Glomerular lipidosis in intercapillary glomerulosclerosis. *Ann. Int. Med.*, 1951, **34**, 592–607.

9. Hartroft, W. S. Histological Studies on Fatty Infiltration of the Liver in Choline-Deficient Rats. In: Sherlock, S. (ed.) Liver Disease. J. & A. Churchill, London, 1951, pp. 90–100.

10. Hartroft, W. S., and Ridout, J. H. Pathogenesis of the cirrhosis produced by choline deficiency. Escape of lipid from fatty hepatic cysts into the biliary and vascular systems. *Am. J. Path.*, 1951, **27**, 951–989.

11. Hartroft, W. S.; Ridout, J. H.; Sellers, E. A., and Best, C. H. Atheromatous changes in aorta, carotid and coronary arteries of choline-deficient rats. *Proc. Soc. Exper. Biol. & Med.*, 1952, **81**, 384–393.

12. Wilgram, G. F.; Hartroft, W. S., and Best, C. H. Dietary choline and the maintenance of the cardiovascular system in rats. *Brit. M. J.*, 1954, **2**, 1–5.

13. Baker, J. R. The histochemical recognition of lipine. *Quart. J. Micr. Sc.*, 1946, **87**, 441–470.

14. McManus, J. F. A. Histological demonstration of mucin after periodic acid. *Nature, London*, 1946, **158**, 202.

15. Gömöri, G. Silver impregnation of reticulum in paraffin sections. *Am. J. Path.*, 1937, **13**, 993–1001.

16. Lillie, R. D. Various oil soluble dyes as fat stains in supersaturated isopropanol technic. *Stain Technol.*, 1944, **19**, 55–58.

17. Perls, M. Nachweis von Eisenoxyd in gewissen Pigmenten. *Virchows Arch. f. path. Anat.*, 1867, **39**, 42–48.

18. Lendrum, A. C. In: Dyke, S. C. (ed.) Recent Advances in Clinical Pathology. J. & A. Churchill, 1947, pp. 457–459.

19. Wilson, W. A trichrome method for staining fat with oil red O in frozen sections. *J. Tech. Methods*, 1950, **31**, 216–220.

20. Wrenshall, G. A., and Hartroft, W. S. Spontaneous diabetes in the dog. *Diabetes*. (In press.)

21. Hartroft, W. S. Accumulation of fat in liver cells and in lipodiastaemata preceding experimental dietary cirrhosis. *Anat. Rec.*, 1950, **106**, 61–87.

22. Hartroft, W. S. Diagnostic significance of fatty cysts in cirrhosis. *A. M. A. Arch. Path.*, 1953, **55**, 63–69.

23. Anderson, G. S. The pathogenesis of diabetic glomerulosclerosis. *J. Path. & Bact.*, 1954, **67**, 241–245.

24. Baxter, J. H., and Ashworth, C. T. Renal lesions in portal cirrhosis. *Arch. Path.*, 1946, **41**, 476–488.

25. Burns, J. L., and Hartroft, W. S. Intraocular hemorrhages in young rats on choline-deficient diets. *Am. J. Ophth.*, 1949, **32**, 79–91.

[*Illustrations follow*]

LEGENDS FOR FIGURES

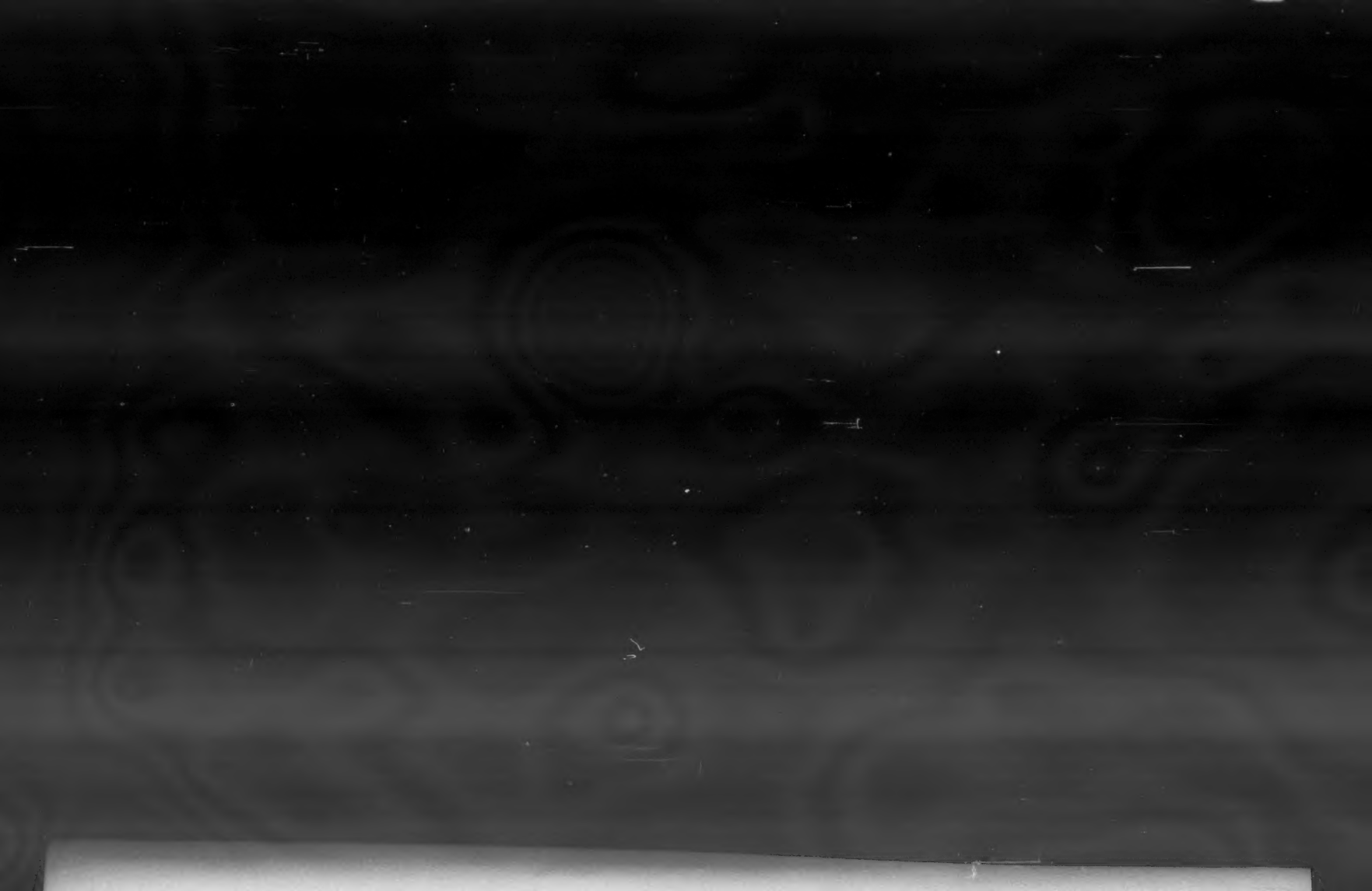
Figures 1 to 8 are of glomeruli from kidneys of rats sacrificed after they had been fed a low-choline diet for periods of 8 to 12 months.

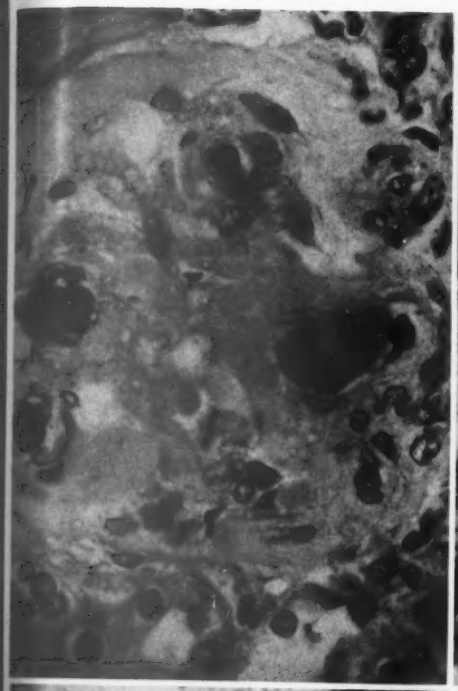
FIG. 1. Fat emboli (dark gray or black) occupy several capillaries in the glomerulus shown. Of note are the thickened capillary walls, and the nuclei of endothelial cells lining the vascular spaces filled with fat. Frozen section stained with oil red O. × 800.

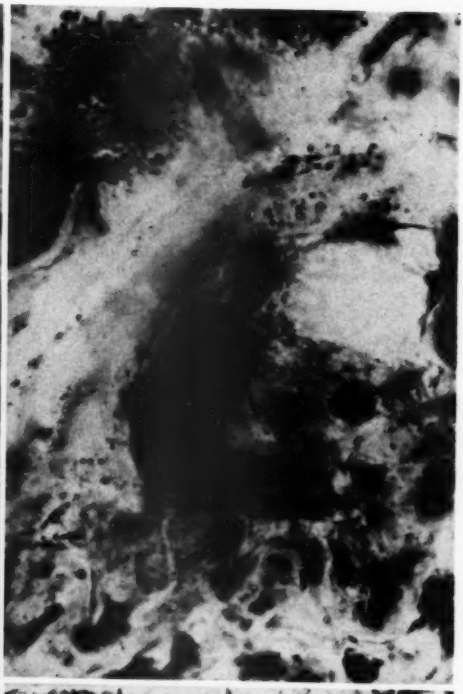
FIG. 2. The capsule of Bowman can be seen at the upper left just below tubule-cells containing fat (black). In the center of the field, a capillary loop filled with fat lies beneath the capsule of Bowman. Plasma-exudate lies between the obstructed loop and the capsule. Frozen section stained with oil red O. × 1000.

FIG. 3. A small portion of Bowman's capsule is in the upper right. The field is almost entirely occupied by a single capillary loop that is thickened and swollen to a severe degree. Endothelial nuclei can still be identified, however. The swollen loop is filled with a mixture of fat (black) and plasma coagulum (gray). Frozen section stained with oil red O. × 1000.

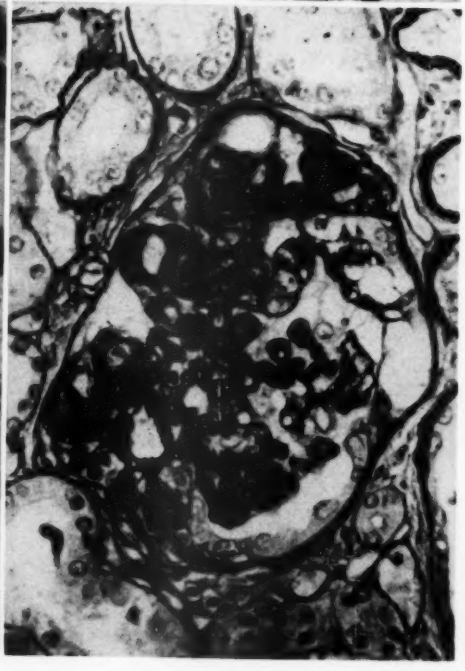
FIG. 4. Thickened, but unobstructed, capillary loops are demonstrated in this section as stained by the PAS method. This section illustrates the appearance in paraffin preparations of glomeruli of the type illustrated in Figures 2 and 3. × 500.







2



4

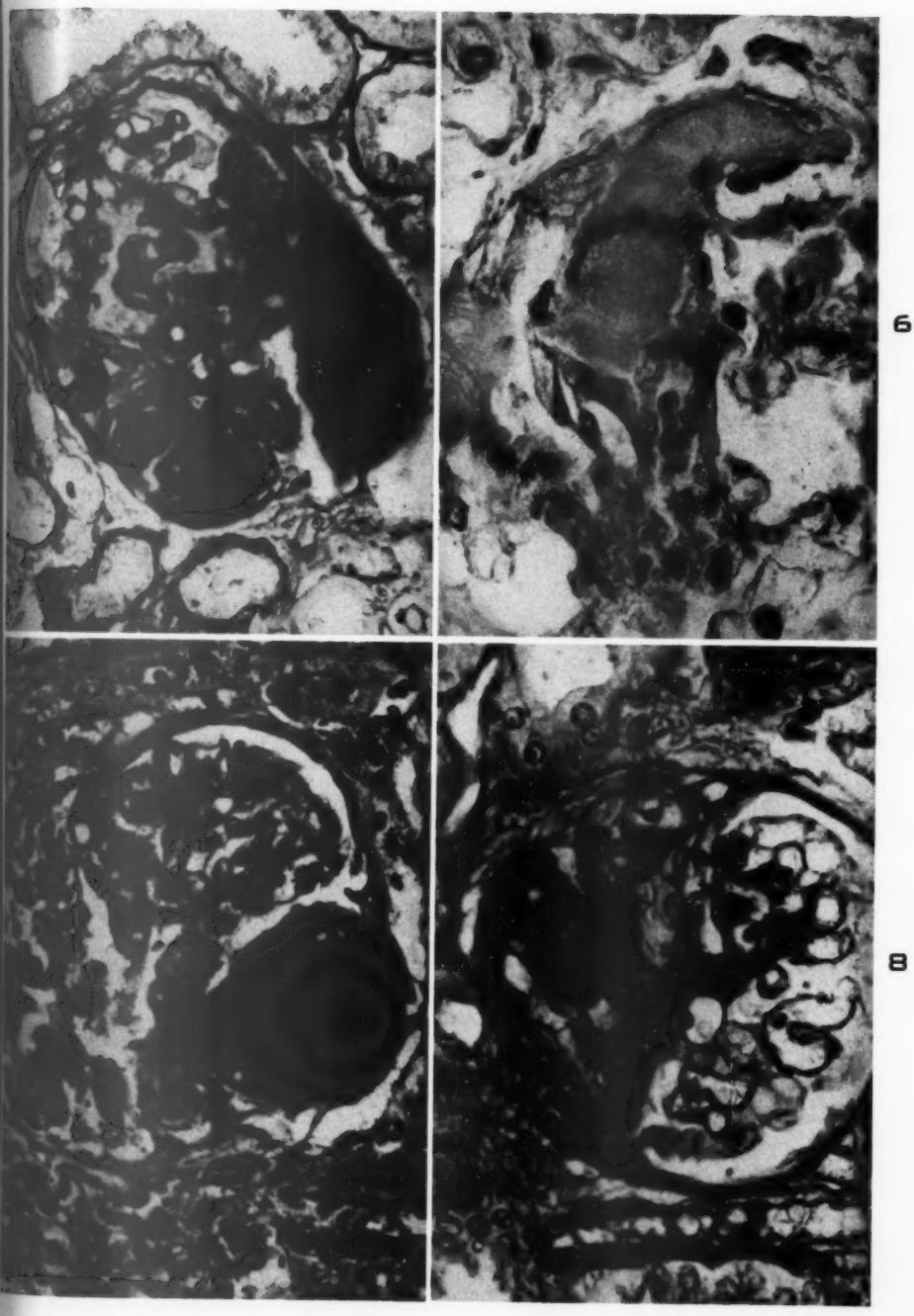
Figures 5 to 8 are from paraffin sections of kidneys of choline-deficient rats.

FIG. 5. Lesions in this glomerulus are at a later stage than those shown in Figure 4. The thickened capillary loops are obstructed by homogeneous coagula that stain faintly by the PAS method. There is an intensely reacting nodule at the right, adherent to the capsule. × 500.

FIG. 6. Nodular sclerosis in a glomerulus of a choline-deficient rat as the lesion appears in sections stained with hematoxylin and eosin. The resemblance to glomerular sclerosis in human diabetic patients is evident. × 800.

FIG. 7. A single, large, focal lesion in an otherwise normal glomerulus is shown stained with hematoxylin and eosin. Endothelial cells have disappeared in the region of the lesion. × 500.

FIG. 8. A lesion of diffuse type in the rat, involving half the glomerulus, is illustrated. PAS stain. × 500.



6

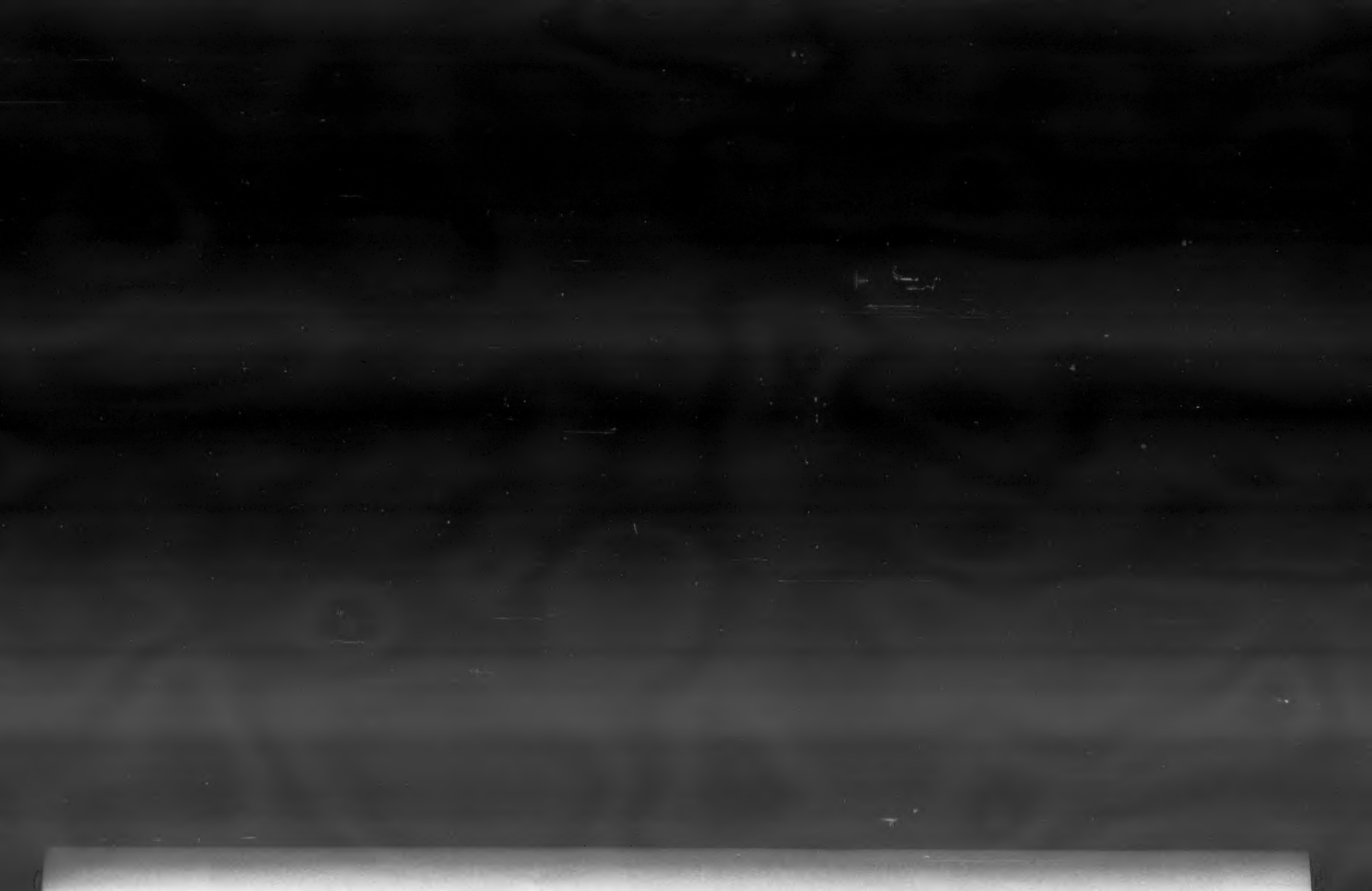
8

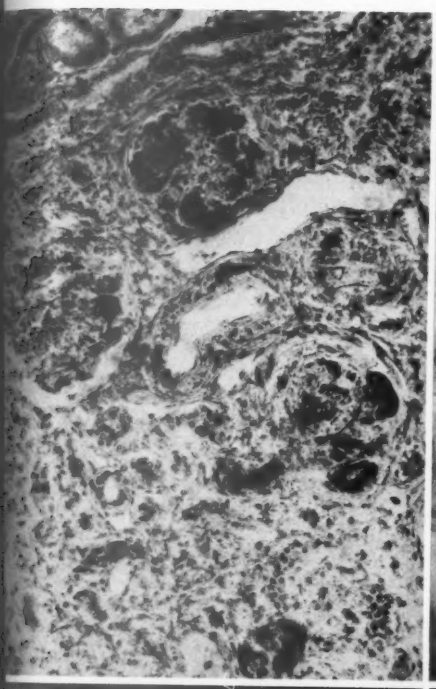
FIG. 9. Frozen section of kidney of diabetic patient stained with oil red O. Fat (black) is present in several portions of the lumina and walls of the vascular tree, including the glomeruli. × 100.

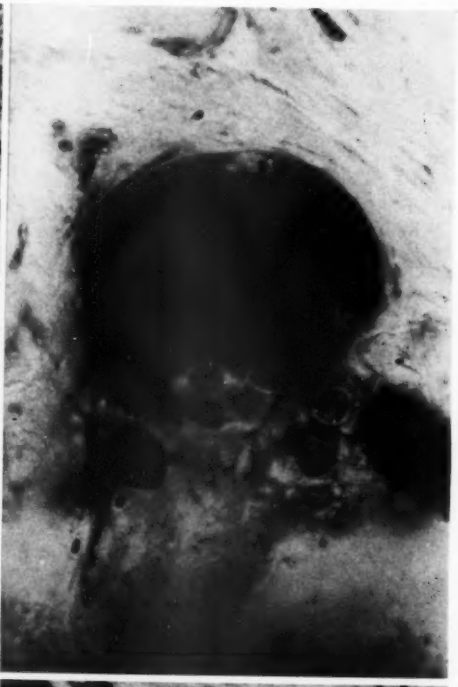
FIG. 10. In this high-power illustration of a single glomerular loop, greatly distended with fat (black) and red blood cells (gray), Bowman's capsule is in the upper right. The endothelial cells of the capillary loop can still be discerned. Frozen sections of kidney of diabetic patient. Oil red O stain. × 1000.

FIG. 11. Fat (black) completely fills and plugs the lumen of this postglomerular arteriole in the kidney of a human diabetic patient. Frozen section. Oil red O stain. × 1000.

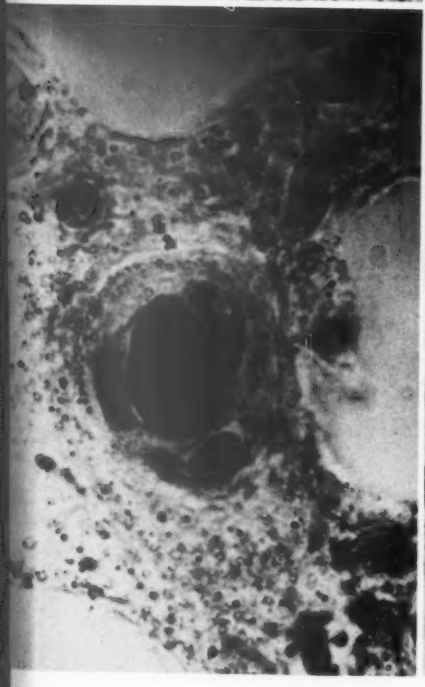
FIG. 12. Fat (black) of somewhat crystalline nature completely fills and plugs this capillary loop which lies immediately beneath Bowman's capsule (upper right). The wall is distended with plasma-exudate. The same process has occurred also in the neighboring capillary loop (upper left). Frozen section of kidney of diabetic patient. Oil red O stain. × 1000.

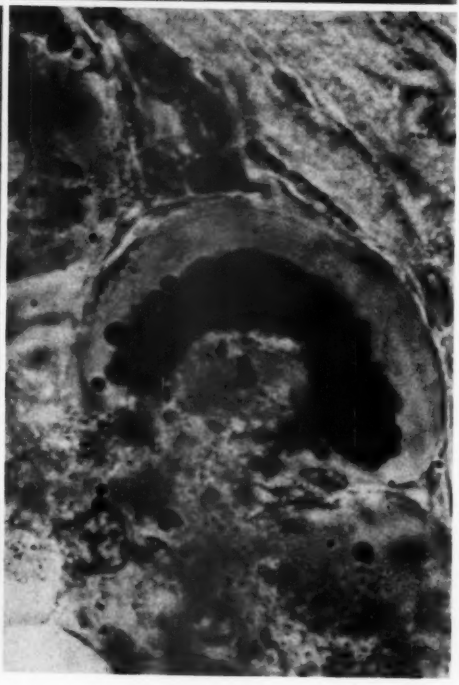






10





12

FAT EMBOLISM IN DIABETIC PATIENTS WITHOUT PHYSICAL TRAUMA *

SIDNEY P. KENT, M.D.
From the Department of Pathology, University of Alabama Medical Center, Birmingham 5, Ala.

Fat embolism is usually thought of as related to trauma, especially traumatic fractures of bones. However, fat embolism has been reported in patients suffering from various diseases unrelated to trauma. Some of the first reported cases of so-called non-traumatic fat embolism were in diabetic patients. Sanders and Hamilton[1] described two such cases in 1879, just 17 years after fat emboli were first described in human beings. Their patients died in diabetic coma and had severe lipemia in addition to fat emboli. Subsequently, a number of similar cases have been reported.[2-4] The lipemia frequently associated with diabetes mellitus was thought by Gröndahl[4] and also by Ebstein[5] to be the source of the fat emboli, the small particles of fat coalescing to form the emboli. Further, Lehman and Moore[6] were able to produce fat emboli in dogs by first inducing lipemia and then administering ether.

Graham[7] described finely divided sudanophilic material in the blood vessels of a diabetic patient who had not experienced trauma. He considered that this finely granular material should be distinguished readily from the true fat emboli believed due to trauma.

Can the fat emboli found in diabetic patients be distinguished from those found in traumatic cases, and does the sustained lipemia often associated with diabetes mellitus predispose such patients to the development of fat emboli? A group of diabetic patients who died from various causes were examined for fat emboli at necropsy and compared with a control group in an attempt to answer these questions.

METHODS AND MATERIAL

In order to eliminate physical trauma as an etiologic agent in so far as possible, patients who had experienced trauma within 3 weeks of death were not included. This period was considered adequate because of the work of Reuter[8] which suggested that fat emboli are eliminated from the circulatory system in 8 to 14 days. For purposes of this report, trauma included traumatic fractures, extensive trauma

* Presented in part at the Fifty-first Annual Meeting of the American Association of Pathologists and Bacteriologists, Philadelphia, April 8, 1954.
Received for publication, August 23, 1954.

to soft tissue, burns, and various surgical procedures such as craniotomy, tracheotomy, laparotomy, thoracotomy, and prostatectomy. Fifty-three non-traumatic, diabetic cases were available. The control group also contained 53 cases. These were consecutive, non-traumatic, non-diabetic, adult cases.

One block of tissue was taken from the lower lobe of each lung and frozen sections were cut at 25 μ. Using Herxheimer's method,[9] two sections from each block were stained with Sudan IV, counterstained with hematoxylin, and mounted in glycerin jelly. Thus, four sections were available for study in each case.

Results

Fat emboli could be differentiated from other sudanophilic material in the lungs by their intravascular position and by their tendency to conform to the lumen of the blood vessel because of the pliability of the fat and arterial pressure. Sudanophilic material other than fat emboli was found in over 90 per cent of the cases. In some, the alveoli were filled with fat. During the process of cutting and staining, this sudanophilic material may be swept into the lumen of the blood vessels. When this occurs, however, it does not take the form of the vessel. Small lobules of adipose tissue occasionally were found adjacent to bronchi. Lehman and McNattin[10] described similar lobules of adipose tissue in the lungs of dogs, but did not find it in a series of human necropsies examined for fat emboli. Sudanophilic material from these isolated lobules of adipose tissue is not likely to be confused with fat emboli if one is aware that adipose tissue occurs in such areas.

Fat emboli were found in 24 of 53 diabetic patients, or in 45.3 per cent. The control series included 11 positive cases, or 20.7 per cent.

The positive cases were classified according to the number of fat emboli found in the sections of lung. When a careful search revealed only a very occasional embolus, the case was designated 1 plus. Cases in which fat emboli were found in several areas of the lung sections were considered 2 plus. In a few cases fat emboli were found in almost every low-power field; these were considered 3 plus. To be classified 4 plus, nearly every low-power field must contain many fat emboli.

When the 24 positive cases in the diabetic group were classified, 19 were 1 plus, 2 were 2 plus, and 3 were 3 plus (Table I). Of the 11 positive cases in the control group, 10 were 1 plus and 1 was 2 plus. None of the cases in either group was considered 4 plus. (The only 4

plus examples that I have seen have been in patients with traumatic fractures.) For easier comparison, the results of classification as to degree of fat embolism are repeated in Table I.

TABLE I

Classification of Positive Cases as to Degree of Involvement

	1 plus	2 plus	3 plus	4 plus
Diabetic group	19	2	3	0
Control group	10	1	0	0

DISCUSSION

Most of the positive cases in the diabetic group as well as in the control group contained few fat emboli. The presence of fat emboli in these cases probably had no clinical significance. Hedren,[8] in examining 60 diabetic patients for fat emboli, found one patient with severe fat embolism; death in that case was attributed to the fat emboli. On the other hand, Warren[11] stated that he had not seen a case of fatal fat embolism among several hundred patients with diabetic coma.

Of the 5 diabetic patients with more abundant fat emboli, 3 cases graded 3 plus and 2 as 2 plus, all were poorly controlled metabolically. Four were admitted and died in diabetic coma. The other patient was admitted in hypoglycemic shock and died a few hours later. These 5 probably had lipemia of some degree, as lipemia is usually more common and more severe in poorly regulated diabetic patients.[12,13] There was, however, no direct evidence that these patients had lipemia, as blood fat determinations were not done. Of course, the higher incidence of fat emboli in this limited series of diabetic patients as compared to the control group does not necessarily implicate lipemic serum as the source of the fat emboli in these patients.

The reports of Hartroft and Ridout[14] and of Durlacher *et al.*[15] suggest "the fatty liver" as another possible source of fat emboli in nontraumatic cases. The livers in the present series were examined in sections stained with Sudan IV. Some degree of fatty change was found in all of the diabetic patients as well as in the patients in the control group. Two types of fatty change were noted. First, there were fatty cysts that could be seen in routine hematoxylin and eosin section. This type was found in 31 of the 53 diabetic patients and in 18 of the 53 control cases. The second type of fatty change consisted of finely granular material in liver cells which could be recognized only

in the fat stains. This was found in all cases and was associated with hepatomegaly in some. The amount of fatty change in each case was estimated as minimal, moderate, or marked. The degree of fatty change was not necessarily in positive correlation with the presence of fat emboli or with the degree of fat embolism. For example, the 3 diabetic patients with the more numerous fat emboli had varying degrees of fatty change in the liver. Marked fatty change was found in the liver of one patient while only a minimal degree was found in the livers of the other 2 patients.

The fat emboli found in the diabetic group as well as in the control group were for the most part well formed. They could not be differentiated from fat emboli found in traumatic cases. A finely divided sudanophilic material was found in the blood vessels of the lungs of 2 of the diabetic patients. However, in no area did the material completely fill the lumina of even small vessels nor did it take the form of the vessels. Similar material was found in the renal vessels of both patients. This is probably material of the same type as Graham[7] described in his case. This may be a manifestation of lipemia, though there was no direct evidence of lipemia in the present cases. Both patients, however, were admitted and died in diabetic coma.

Summary

The lungs of 53 diabetic patients who had not received known physical trauma within 3 weeks of death were examined for fat emboli. Fifty-three consecutive, non-traumatic, non-diabetic cases were used as controls. Fat emboli were fairly common in the diabetic group as well as the control group, but the percentage of positive cases in the diabetic group was considerably higher than in the control group. Most of the positive cases were of minimal degree and probably had little clinical significance.

The emboli found in the diabetic patients could not be distinguished from those found in traumatic cases.

The pathogenesis of non-traumatic fat embolism is discussed in relation to lipemia and fatty change in the liver.

REFERENCES

1. Sanders, W. R., and Hamilton, D. J. Lipaemia and fat embolism in the fatal dyspnoea and coma of diabetes. *Edinburgh M. J.*, 1879–80, **25**, 47–57.
2. Starr, L. Lipaemia and fat embolism in diabetes mellitus. *M. Rec.*, 1880, **17**, 477–481.
3. Hedren, G. Fettembolie och diabetish lipemi. *Svenska läk.-sällsk. handl.*, 1916, **42**, 933–946.

4. Gröndahl, N. B. Untersuchungen über Fettembolie. *Deutsche Ztschr. f. Chir.*, 1911, 111, 56–124.

5. Ebstein, W. Beitrag zur Lehre von der Lipaemie, der Fett-Embolie und der Fett-Thrombose bei der Zuckerkrankheit. *Virchows Arch. f. path. Anat.*, 1899, 155, 571–586.

6. Lehman, E. P., and Moore, R. M. Fat embolism including experimental production without trauma. *Arch. Surg.*, 1927, 14, 621–662.

7. Graham, G. S. Fat embolism: report of a case and of experiments on animals. *J. M. Research*, 1907, 16, 459–482.

8. Reuter, W. Experimentelle Untersuchungen über Fettembolie. *Frankfurt. Ztschr. f. Path.*, 1915, 17, 205–217.

9. Mallory, F. B. Pathological Technique. W. B. Saunders Co., Philadelphia, 1938, ed. 1, p. 118.

10. Lehman, E. P., and McNattin, R. F. Fat embolism. II. Incidence at postmortem. *Arch. Surg.*, 1928, 17, 179–189.

11. Warren, S. Fat embolism. *Am. J. Path.*, 1946, 22, 69–87.

12. Bodansky, M., and Bodansky, O. Biochemistry of Disease. Macmillian Co., New York, 1952, ed. 2, pp. 506–507.

13. Rubin, S. H. The plasma and red blood cell lipids in persistent (diabetic) lipemia and in transient (alimentary) lipemia. *J. Biol. Chem.*, 1939, 131, 691–702.

14. Hartroft, W. S., and Ridout, J. H. Pathogenesis of the cirrhosis produced by choline deficiency; escape of lipid from fatty hepatic cysts into the biliary and vascular systems. *Am. J. Path.*, 1951, 27, 951–989.

15. Durlacher, S. H.; Meier, J. R.; Fisher, R. S., and Lovitt, W. V., Jr. Sudden death due to pulmonary fat embolism in persons with alcoholic fatty liver. (Abstract) *Am. J. Path.*, 1954, 30, 633–634.

HISTOGENESIS OF HEPATIC CIRRHOSIS STUDIED BY THE THREE-DIMENSIONAL APPROACH *

HANS POPPER, M.D., and HANS ELIAS, Ph.D.

From the Hektoen Institute for Medical Research and the Department of Pathology of Cook County Hospital (Dr. Popper) and the Department of Anatomy, Chicago Medical School (Dr. Elias), Chicago, Ill.

The forces that transform a normal liver into a cirrhotic organ are not clearly understood. Many hypotheses have been offered.[1-6] The most acceptable description of cirrhosis is that of an altered reconstruction of the lobular pattern; however, the dynamics of the altered reconstruction are still not fully established. The recent re-examination of the structure of the normal liver based on three-dimensional analysis[7] and a statistico-geometric method[8] suggested the utilization of the same principle in the approach to the study of the cirrhotic liver. In the continuation of investigations devoted to the vascular supply in cirrhosis,[9] the structural alterations of parenchyma and connective tissue have been analyzed. Whether the connective tissue alterations are considered the primary process in cirrhosis[10] or as secondary to parenchymal impairment,[2] these alterations are in the morphologic foreground and received, therefore, the main attention in the study to be reported.

MATERIALS AND METHODS

Necropsy material demonstrating various types of cirrhosis was investigated. The material was fixed in formalin and cut in either single or serial paraffin sections. In addition to routine hematoxylin and eosin stains used primarily for diagnosis, van Gieson's stain with or without nuclear stain was employed. Mallory's aniline blue stain and the silver impregnation method of Gomori were applied also. Single sections, cut in three directions perpendicular to one another, were subjected to statistico-geometric analysis.[8] Reconstruction from serial sections was made with the help of either glass plates[11,12] or wax plates. In some instances models were made by photographing serial sections stained by van Gieson's method without nuclear stain on negative films. Prints were made on lantern slide plates which later were stacked to give an authentic three-dimensional image of the collagenous connective tissue. In this study, collagenous connective

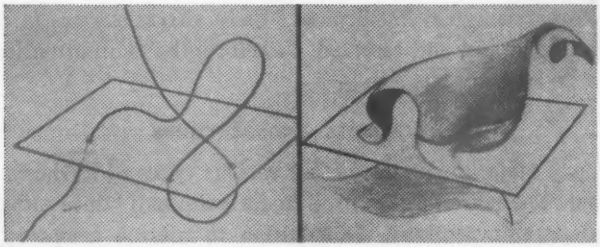
* Supported by grants from the U.S. Public Health Service and from the Dr. Jerome D. Solomon Memorial Research Foundation.

Received for publication, August 9, 1954.

tissue is defined as the material stained red by van Gieson's method. It is not necessarily identical with collagen as defined chemically or with collagen fibers as defined by characteristic periodicity under the electron microscope. Further investigations will have to clarify the relation of argyrophilic (reticulum) fibers to collagenous material.[13,14] The factual observations presented frequently were arrived at by elimination or acceptance of alternate hypotheses. Descriptions of these hypotheses would lengthen this report cumbersomely. Detailed descriptions are therefore presented in a separate study[15] primarily devoted to histo-mechanical considerations of the formation of cirrhosis.

Connective Tissue: General Observations

By the application of statistico-geometric criteria, evidence has been obtained[8] that the collagenous connective tissue in many cases of cirrhosis consists mostly of membranes rather than of fibers. A fiber, as a structure of mainly one dimensional extension, appears in a histologic section as a dot (Text-fig. 1) or as a very short line or



Text-fig. 1. Drawing demonstrating appearance of a fiber or of a membrane in a histologic section.

"comma." The length of the "comma" depends on the angle of inclination between fiber and cutting plane and on the thickness of the section. Short commas and dots are far more numerous than long lines when masses of fibers are sectioned. If long lines prevail in the section, the presence of membranes as predominantly two-dimensional structures, such as a sheet of paper, must be assumed, for almost any plane that intersects a membrane will produce a line. Several membranes may aggregate to form a thicker septum which thus represents a thicker, but still flat, mainly two-dimensional, structure.

Processes Resulting in the Formation of Cirrhosis

Formation of cirrhosis may result from several processes described under the following headings: (1) collapse following massive and

submassive necrosis; (2) portal and periportal inflammation; (3) central toxic necrosis; (4) passive congestion; (5) fatty metamorphosis, and (6) pericholangiolitis (inflammation around the smallest bile ducts). In all of the processes, parenchymal nodules eventually form by dissection from the lobular parenchyma. These nodules may be composed of several lobules or of parts of them, as in coarse nodular cirrhosis[1,5]; or the nodules may be parts of one original lobule. The process of the formation of the second type of small nodule will be discussed under portal and periportal inflammation, although it applies to other forms as well.

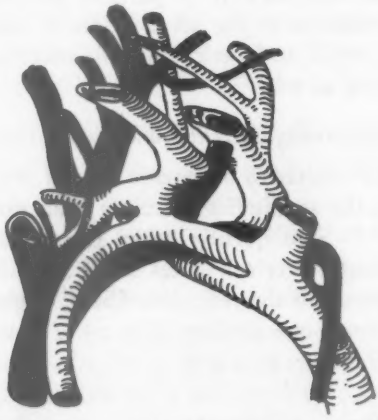
Collapse Following Massive and Submassive Necrosis

Following massive necrosis of hepatic cells of entire liver lobules or groups of lobules, the argentaffin reticulum framework at first remains intact (Fig. 1).[16,17] Subsequently, when regenerating liver cells fail to replace the necrotic liver cell plates or the surrounding exudate, the framework collapses and the reticulum fibers, originally separated by liver cell plates, touch one another (Fig. 2). The argentaffin network appears denser and surrounds only a few slits; but there is no convincing evidence of new formation of reticulum fibers.

Before the collapse, a delicate, slightly fuchsinophilic material is noted between the necrotic liver cells. On high contrast films the material has a fine fibrillar structure (Fig. 3). After the collapse, a few collagenous membranes become visible (Fig. 4). They appear suspended in the meshes of the reticulum fibers and are only exceptionally independent of them. They are without question newly formed connective tissue, although fibroblasts are hardly noted (Fig. 5). The arrangement is similar to that described[18] for the zona glomerulosa of the adrenal cortex.

When entire liver lobules collapse, the portal and hepatic canals become approximated, resulting in relatively large areas of connective tissue, vascularized by some of the old sinusoids (Fig. 6). The portal triads and the central hepatic veins appear in normal arrangement, but the distance between them is considerably shorter than 0.5 mm. which is the average radius of a healthy liver lobule. This is seen in the grossly visible large scars characteristic of the post-necrotic or toxic cirrhosis.[1,5,19-21] Reconstruction of such an area reveals that the normal characteristic interdigitation between the portal and hepatic venous tree[22,23] is maintained (Fig. 7); however, in contrast to the norm, the angle of branching of portal and hepatic vessels becomes sharply acute (Text-fig. 2).

In the collapsed area the preservation of the basic architecture is indicated also by the characteristic difference of the collagenous connective tissue in the collapsed lobule itself, which is membranous in character, from the thick fibers and fibrous bundles of the portal triads and the adventitia of the central veins, which appear on cut sections as dots or commas (Fig. 7).



Text-fig. 2. Reconstruction of portal canals (white) and hepatic canals (black) in a collapsed area after massive necrosis. The approximation of both systems and the acute angulation with maintenance of original interdigitation may be noted. The arch on the bottom was filled out by surviving liver tissue.

This process of massive necrosis and collapse may set in motion other processes. A rapidly collapsing area acts as a partial vacuum[9] and thus exerts centripetal traction upon the surrounding tissue which is not massively necrotic. This results in lines or planes of stress (Fig. 8) which are arranged radially about the area of collapse. Therefore, the liver plates become stretched in the direction of the traction and are consequently flattened transversely. At the same time the reticular framework is distorted but not broken, and the sinusoids are dilated. The liver cells atrophy in this location and disappear. This results in the appearance of a parenchymal fissure. Collagenous membranes develop in the persisting reticulum framework. Numerous membranes developing in a fissure may aggregate to form a septum. This septum traverses the parenchyma surrounding the collapsed area without relation to the lobular architecture. Development of fissures from stress followed by septum formation has been noted also in the livers of pigs.[24]

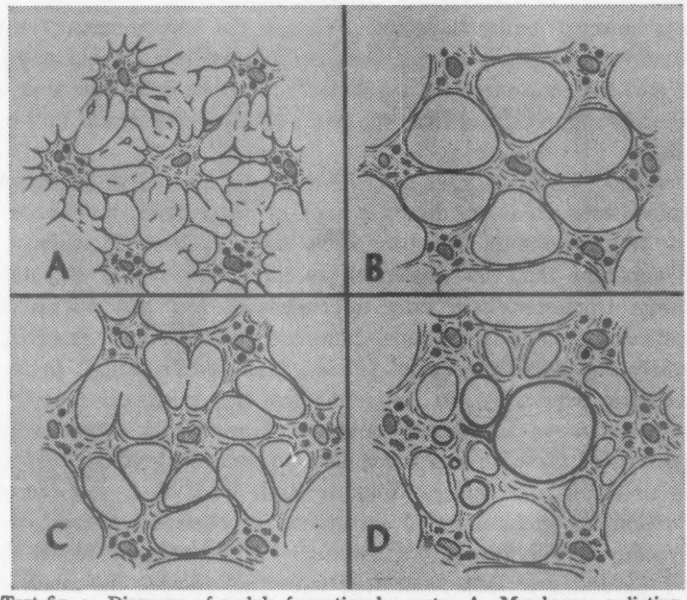
In submassive collapse,[25] only part of a lobule or parts of neighboring lobules become necrotic (Fig. 9). The collapsed portions reveal changes similer to those in massive collapse, whereas the non-necrotic portions are traversed by septa resulting from fissures. Regeneration and re-arrangement of liver cell plates, moreover, produce nodule formation, as will be discussed.

In principle, the process is similar whether massive or submassive necrosis occurs in a previously cirrhotic parenchyma (secondary collapse); however, under these circumstances the approximated central fields and portal triads do not have the normal relationship to each other and the connective tissue membranes of the collapsed area are not sharply differentiated from the fibers of the portal triads and central fields.

Comment. Cirrhosis after collapse following massive or submassive necrosis results from the disappearance of all or many liver cells of one or several lobules. The connective tissue increase in collapse is more apparent than real inasmuch as the fibers of the argentaffin reticulum framework are only approximated and not augmented. Delicate collagenous membranes, however, are perhaps formed from extremely fine fibrillar material (giving "collagen" staining) between the necrotic liver cells. In the connective tissue masses the vessels are approximated. In primary collapse of previously normal tissue, the pattern of these vessels is regular. In secondary collapse of cirrhotic tissue, it is irregular. Collapse itself leads to a post-necrotic scar which, as such, does not represent cirrhosis formation. Such scars occur in syphilis (hepar lobatum) or in atrophy of the left lobe.[26] Several reactions associated with the collapse do cause cirrhosis. The first is the formation of fissures in the non-massive necrotic tissue. In these fissures connective tissue septa form with a few persisting vessels, thus dissecting the lobules also outside the collapsed area. Second, in submassive collapse,[25] lobular fragments form nodules which either had been part of a lobule or, if larger, are composed of several lobules. The smaller the nodules[19-21] and the more acute the necrosis, the more intensive are the regenerative changes (to be discussed). Third, acute necrosis incites portal and periportal inflammation, potentially resulting in septum formation. These three processes are more intense in the rather rapid type of collapse resulting from viral infection or intoxications. The post-necrotic scarring, therefore, in these conditions, usually is associated with cirrhotic changes in the rest of the liver.

Portal and Periportal Inflammation

Various types of inflammation in the portal triads, as produced by bacterial infection, granulomatous or parasitic diseases, iron deposition in hemochromatosis, and possibly also in later stages of viral hepatitis, are associated with increase of collagenous connective tissue in the portal triads and the surrounding periportal area. The inflammation is often associated with destruction of the limiting plate[7] and



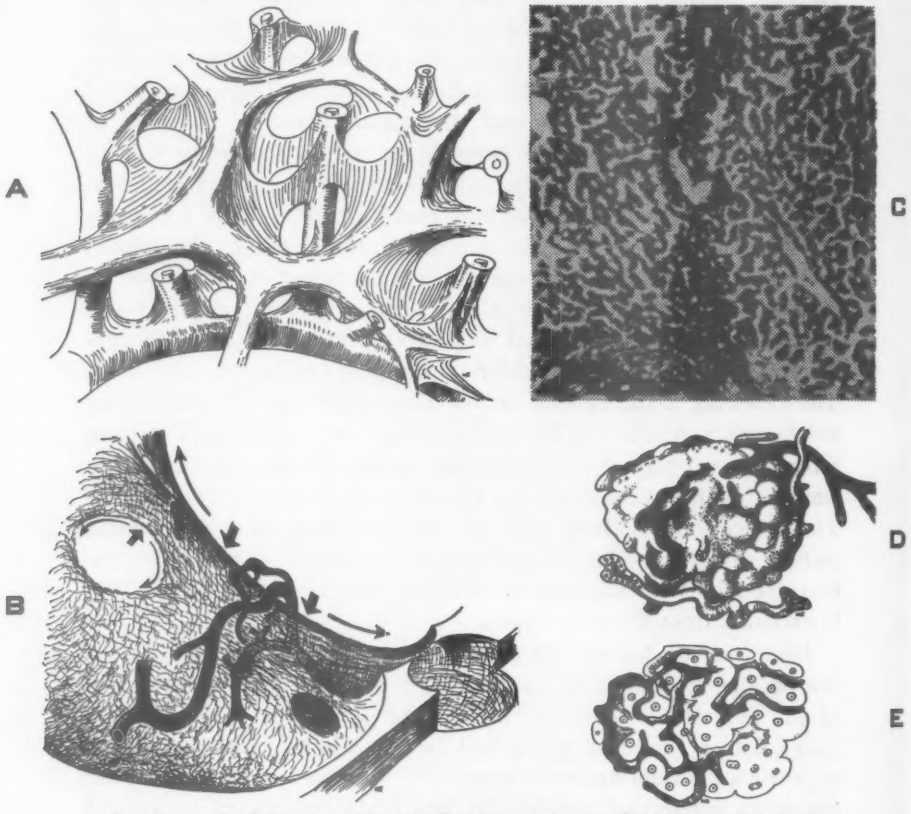
Text-fig. 3. Diagrams of nodule formation by septa. A. Membranes radiating from portal and central fields. B. Membranous tracts aggregate to form septa which subdivide the lobule. (Text-figs. 3A and 3B are reproduced by permission of the Josiah Macy, Jr. Foundation from *Transactions of the 11th Conference on Liver Injury*, 1952, page 181.) C. Further subdivision of lobular fragments (nodules) by septa. D. Regenerating nodules obscure original architecture.

a strand-like infiltration of the periportal area by inflammatory cells (Fig. 10). In early stages this is associated with formation of delicate collagenous membranes found between the liver cell plates radiating from the portal canals (Fig. 11) and acting as reinforcements of the reticulum fiber framework in the tissue space. Membranes develop by adhesion of smallest "micromembranes" (Fig. 12). The membranes finally touch one another when liver cells disappear between them and thus septa are formed which are recognized with low magnification. These septa extend from the portal triads and assume various shapes as seen in three-dimensional reconstruction (Fig. 13).

The septa developed by selection of micromembranes originate in the forks of the portal canals. The sinusoids located in the developing septum become incorporated in it, some of them persisting as blood-carrying channels. They lose their sinusoidal character and become little veins as described by Moschcowitz[27]; but they are not necessarily associated with inflammation and granulation tissue formation. Eventually stronger tracts of membranes develop. They may extend from one portal triad to the next (periportal "fibrosis") but some of them extend into the lobular parenchyma, subdividing it as seen in the reconstructed model (Fig. 13). If the septa reach the central field (Fig. 15), the lobule is at least partly cut off to form a primary nodule. At the same time the vessels incorporated into the septum (Fig. 14) produce a porto-hepatic venous shunt as described in human[9] and experimental cirrhosis.[28] Owing to these shunts which may be considered as internal Eck fistulas, blood by-passes the parenchyma, which is placed under unfavorable circulatory conditions. The resulting hepatocellular injury may aggravate the pre-existing inflammatory reaction in the portal triads; new membranes forming around the primary septa develop into secondary septa which in turn lead to further subdivision of the primary nodules (Text-fig. 3). These secondary nodules grow at variable speeds and an irregular picture develops. With the processes characteristic of nodule formation, the lobular architecture is abolished and the original central vein is excluded from the parenchyma.

Mechanics of Septum Formation. If the central and portal veins ran parallel to each other, as depicted in the well known stereogram of the liver lobule in Braus' book,[29] the primary nodules would be long columns; however, portal and hepatic venous branches cross at approximately right angles in space.[7,22,23] This makes it necessary for the septa connecting the hepatic and portal canals to be twisted (Text-fig. 4A), and this, in turn, influences their shape. The septa cannot isolate the nodules completely from one another; however, each septum has two free edges. These edges are often indistinct, but far more frequently they are sharply outlined. Upon reconstruction (Text-fig. 4B), when the free edges are sharp, they have the shape of a concave line. Such a shape will be developed if a line firmly attached at both ends (slender arrows, Text-fig. 4B) is exposed to a transverse resistance (stout arrows, Text-fig. 4B). The points of attachment of primary septa are at the portal and hepatic canals. The points of attachment of secondary septa are at the primary septa. The resistance is offered by the parenchyma which prevents the straightening of

the line. As a rule, the septa are flat, reflecting the tension to which they are exposed. This tension is due to expansion of the parenchyma which pushes the points of insertion of the septa apart, or it is due to



Text-fig. 4. A. Stereogram. The interdigitation of the portal and hepatic venous tree causes: (a) torsion of the septa connecting portal and central canals and (b) free edges of the septa, preventing complete separation of the nodules from each other. (Text-fig. 4A is reproduced by permission of the Josiah Macy, Jr. Foundation from *Transactions of the 11th Conference on Liver Injury*, 1952, page 191.) B. Reconstruction of septum with free edge distorting efferent venule which becomes tortuous. Slender arrows indicate traction; stout arrows, tissue resistance. C. Crooked septum indicative of lack of tension and efferent vessels. Mallory's aniline blue stain. × 80. D and E. Reconstruction of small regenerating nodule: D, surface appearance; E, cut surface. All cells are in contact with sinusoids. In D, connection with a ductule is shown.

contraction of the membranes forming the septa; or it may be due to both. The often noted wavy character of such membranes (Fig. 12) may be evidence of contraction.

Processes Inherent in Nodule Formation. When part of a lobule

becomes separated from the rest of the parenchyma, several characteristic changes develop.

1. The liver plates are rearranged owing to alteration of the sinusoidal blood flow. The concentric direction toward the central vein of the lobule is abandoned, and after some irregular arrangement, a new concentric arrangement is resumed toward the center of the nodule (Fig. 16, lower right; Fig. 17).

2. New efferent venules develop from intranodular sinusoids; these efferent venules are tortuous, primarily because of their origin from sinusoids, and secondarily, because of distortion by the advancing septa (Text-figs. 4B and 4C).

3. The parenchyma undergoes regeneration, partly owing to the proximity to liver cell destruction and partly owing to humoral effects in the presence of destruction of hepatic parenchyma anywhere in the liver.[30] This regeneration produces plates two or more cells thick (Fig. 17) throughout the smaller nodules. In larger nodules such plates are seen near the periphery of the lobules; in the center, the plates are one cell in thickness (Fig. 18). Rapidly regenerating nodules compress the surrounding connective tissue which thus passively may assume the shape of a septum; or the membranes may be disrupted and transformed into palisades of parallel fibers. The veins around such nodules are compressed. The tributaries of the hepatic veins are flattened since they have only a thin connective tissue cover.

4. Isolated cells or cell groups may become trapped in the connective tissue. Some of them are fat-containing and may even develop into fatty cysts.[31] These isolated cells or cell groups may become atrophic; they may die, or they may start regenerating, forming a group of cells not separated by sinusoids (Fig. 21). Eventually, a well vascularized small nodule may form from such trapped cells. The nodule will contain at its periphery plates of several cell thicknesses (Text-fig. 4D). At a later stage (Fig. 19) the plates will become thinner toward the center; in the larger nodules, they become one cell in thickness. Circulatory disturbances may lead to atrophic thinning and even necrosis of cells in the center of the nodule (Fig. 20).

5. On the border of the lobules the liver cell plates may be transformed into bile ducts of the smallest size or cholangioles (Fig. 22). In accordance with the embryologic observation[32] that cholangioles originate from liver cells rather than vice versa, the connection of these proliferating cells with the biliary duct system has often been observed[33] and demonstrated by reconstruction (Text-fig. 4D).

Comment. The development of cirrhosis as a result of periportal

inflammation starts with a radiating periportal collagenous membrane formation (membranosis), a process commonly seen under the most diverse circumstances. The histogenesis of this membranosis requires further elucidation. In view of the absence of fibroblasts, it is still possible to defend the old theory[10,34] that plasma protein which has escaped into the tissue spaces (serous hepatitis) is the stimulus and furnishes the material for the collagenous membranes. Recently such a process occurring in children in Jamaica as the result of either malnutrition and/or toxicity was called collagenosis[35]; however, inasmuch as collagen differs chemically from plasma proteins, additional histochemical studies concerning membrane formation in the liver and regarding the influence of liver cell breakdown products on membrane formation are required.

This radiating periportal membranosis usually remains stationary. Sometimes, for reasons not obvious, membranes may aggregate and the intervening liver cells disappear, so that thicker septa form, extending into the lobular parenchyma. The sheet-like septa may connect portal triads with each other (perilobular "fibrosis"), but may extend into the lobular parenchyma itself and connect portal with hepatic fields.

Inasmuch as the portal and hepatic venous trees interdigitate in space, they cut out portions of the lobule; eventually secondary septa increase the subdivision. Nodules of various sizes and shapes are formed. Rearrangement of the liver cell plates, formation of new efferent venules, and irregular regeneration contribute to the abolition of the lobular architecture. This is aggravated by a counteraction between the nodules expanding as a result of regeneration and the tension and contraction of the connective tissue of the septa. It should be emphasized that in the cirrhotic liver, expansion of the regenerating parenchyma may exceed the resistance offered by the usually tough collagenous connective tissue (Text-fig. 4C).

Central Toxic Necrosis

After central necrosis and collapse of the framework in the central zone, membranes may develop which radiate from the central vein. Some membranes become aggregated to produce septa that connect hepatic canals with each other, but eventually also hepatic with portal canals. These septa thus subdivide the lobule. Central toxic hepatic necrosis usually is associated with periportal inflammation and subsequent radiating membranosis. Septa originating from the portal triads interconnect with those from the central fields to produce a similar

dissection of the lobule with nodule formation occurring in the manner described.

Comment. Single toxic insults are usually not followed by septum formation. Repeated attacks are required[6] as seen, for instance, in carbon tetrachloride[36] or bromobenzene[37] intoxication.

Passive Congestion

Membrane and septum formation following centrolobular necrosis in acute passive congestion do not differ from those following toxic central necrosis.[38] Initially, owing to a reduction of the normal portohepatic blood pressure gradient,[39] the liver cell plates and the sinusoids rearrange themselves to radiate from the portal canals. Then the areas of necrosis connect neighboring central veins in a bridge-like fashion, so that the periportal regions appear surrounded by continuous red, congested areas and the lobular pattern seems inverted. In longer standing congestion, radiating membranosis develops around the central vein, sometimes extending into the bridges of necrosis which connect the central veins.[40] This does not yet constitute cirrhosis, however. Eventually in rare instances, a cardiac cirrhosis develops. In such cases the lobular architecture is distorted when septa have come to connect central and portal fields. This may be facilitated if periportal inflammation complicates the congestion.

Comment. The processes of development of the rare cardiac cirrhosis[41] are similar to those following central toxic necrosis.

Fatty Metamorphosis

Connective tissue accumulation in the fatty liver preceding and possibly leading to the formation of cirrhosis may occur as a result of several processes:

1. In livers with advanced fatty metamorphosis, fissures are frequently noted (Fig. 23). The constancy of their appearance and their direction, which is not related to that of the microtome knife, speaks against their being artifacts. They range from 10 to 25 μ in width, often radiate from a portal triad, and may traverse long distances. As straight lines in sections, they represent flat fissures in three-dimensional space. When two hepatic regions break apart owing to uneven expansion, the border of these regions produces a fissure. The following processes may be responsible for uneven expansion:

(a) Foci of necrosis occur frequently in the fatty liver and, as a result of contraction, they may be separated by fissures from the intact or even regenerating tissue.

(b) Regeneration with development of fat-free plates two-cells thick may occur in some hepatic territories following recovery from fatty degeneration (Fig. 24). This may take place particularly in the periportal areas which are in general more likely to regenerate. Also formation of intensely regenerating nodules induces fissures in their vicinity, in these instances as a result of expansion.

(c) In chronic fatty metamorphosis some areas temporarily recover, especially in the common nutritional forms when periods of poor nutrition may alternate with those of better nutrition. More fat and larger droplets are sometimes noted on one side of the fissure than on the other (Figs. 23 and 27). This reflects different rates of fat deposition resulting in different rates of expansion.

Within the fissures liver cells disappear and collagenous membranes or fibers develop which aggregate to form septa (Fig. 25). In the sections the completed septa appear as straight lines without tension (Fig. 26), which originate in most instances in the portal triads.

In prolonged fatty metamorphosis, the fat droplets of several liver cells coalesce to form fatty cysts as demonstrated by Hartroft and Ridout[31] in choline-deficient rats (Fig. 28). As they described it, after disappearance of the fat the shells of the fatty cysts collapse and give rise to connective tissue membranes. In addition, collagenous membranes (Fig. 29) or fibers (Fig. 30) develop in the vicinity of the fatty cysts but independent of them in regenerating masses of liver cells, forming plates from two to several cells thick.

2. Around the frequent areas of periportal inflammation in fatty cirrhosis, membranes form following the pattern described under *periportal inflammation*. These membranes eventually result in dissection of the lobule (Fig. 31). Membrane formation around intralobular foci of necrosis may also contribute (Fig. 32).

The morphogenesis of the septa in fatty cirrhosis is not easily recognized in the advanced stage but becomes apparent in the transition of fatty liver into fatty cirrhosis.[42] Septa resulting from inflammation and developing septa in fissures are of greater importance than those developing around fatty cysts. The septa of different origin, however, have the same effect in distorting the lobular architecture. Reconstruction shows that they do not separate the nodules completely from one another (Fig. 33).

Comment. Multiple pathways have been described to explain the development of cirrhosis from fatty liver. Some investigators[6,43,44] have assumed that the deposition of fat itself causes the fibrosis, whereas others have expressed doubt as to whether fatty metamor-

phosis, as such, induces cirrhosis,[45-47] and have pointed out that in man, fatty metamorphosis may persist for a long time without producing anything more than portal "stellate" fibrosis. In the malignant malnutrition of kwashiorkor, for instance, cirrhosis develops after a long interval in which the liver is fat-free.[45] Similarly, in ethionine intoxication of the rat, fatty liver develops but disappears again, and fibrotic and cirrhotic transformation follows after a prolonged fat-free period.[48]

Of the pathways illustrated here by three-dimensional reconstruction, only one is related to the fat deposition directly; namely, the membrane formation following the collapse of fatty cysts as described by Hartroft.[31,43] This appears to be the most important process in choline-deficient rats. In man, however, this seems to be in the background compared to two other processes which are only indirectly related to fat deposition. One is represented by the septa which develop from inflammatory changes in and around the portal triads and in the parenchyma, often associated with necrosis and regeneration. These inflammatory and necrotizing changes occur more commonly in the fatty liver than in the normal liver, and are a reflection that the fatty liver is more sensitive to infections or other injuries.[42] The other is the development of fissures due to stress resulting from uneven tissue turgor caused by variation in fat deposition, necrosis, and intralobular periportal and nodular regeneration. This is followed by formation of membranes and straight septa. Variations in the structure of the liver in time and space, therefore, appear more important than the permanency of the fat deposition and explain why fatty metamorphosis does not necessarily lead to cirrhosis in man.

In agreement with clinical observations, it appears that episodes of necrosis associated with infections or other injuries anywhere in the body, as well as subsequent regeneration and variation in the degree of nutritional disturbance, again associated with subsequent regeneration, are of greater importance in man in the transition into cirrhosis than is the fat deposition itself.[42]

Pericholangiolitis

Chronic inflammatory changes in and around the cholangioles are associated with connective tissue proliferation. The connecting pieces between the bile capillaries and the interlobular bile ducts in the portal triads have been designated as septal ducts[49,50] or canals of Hering, or cholangioles or ductules.[30] Ductules are found in greatest number in the normal liver around the portal triads, but they also pervade the

hepatic parenchyma as first described by Eberth[51] and subsequently by Clara.[52] They do not end blindly but form loops anastomosing with other intralobular ductules deep in the lobular parenchyma.[7] For a considerable distance, they are accompanied by intralobular arterioles or arterial capillaries and, occasionally, by an extremely thin lymph vessel which can be demonstrated by India ink injection.[7] These intralobular conduits are surrounded by a common delicate sheet of connective tissue which constitutes their common adventitia and which itself is arranged in filamentous form. Normally, the intralobular ductules are small, less than 4 μ in diameter, and can be seen only at high magnification. Sometimes, however, they become enlarged up to 20 μ in diameter (Fig. 34) and are easily visible. The causes of this transformation, sometimes associated with jaundice, are as yet unknown.

Inflammatory cellular exudate may accumulate around the periportal and intralobular ductules. Around the latter, it is found within the described adventitial sheets, and it therefore cannot be decided whether the lesion represents a pericholangiolitic or a perilymphangitic infiltration. In prolonged cases of extrahepatic as well as of intrahepatic cholestasis (cholangiolitis),[53-56] these changes are especially apparent, and eventually sometimes rather coarse connective tissue fibers appear in the pre-existing connective tissue trabeculae which thus become more conspicuous (Figs. 35 and 36). In reconstruction, the trabeculae appear curved and cylindriform (Fig. 38). Even if these trabeculae are numerous and liver cell groups appear separated from one another by them (Fig. 37), the lobular pattern is not destroyed. Eventually, however, membranes appear first around the portal triads (perilobular fibrosis) and subsequently connect the portal triads with the trabeculae and dissect the lobules as they extend into them (Fig. 39).

Comment. The pericholangiolitic type of fibrosis, even if conspicuous, differs from the previously described cirrhosis in that cylindriform trabeculae rather than flat septa develop, at least in the pure form. These trabeculae traverse the lobular parenchyma, initially representing a reinforcement of the common adventitia of the intralobular arterioles, ductules, and lymphatics. With further proliferation of the ductules, this cylindriform system may become rather dense; nevertheless, the lobules are not dissected. In view of the intact lobular pattern, this lesion does not represent cirrhosis in the sense of the definition given and could be called pericholangiolitic pseudo-cirrhosis. It is not, as a rule, associated with portal hypertension nor with

ascites. Many of these pure lesions are associated with severe jaundice and resemble primary biliary cirrhosis or Hanot's[5] or cholangiotoxic cirrhosis.[10] Only in later stages of this lesion do septa form, possibly owing to a complicating bacterial infection of the portal triads or other causes of necrosis. This is associated with dissection of the lobules and produces, in turn, portal hypertension as is usually seen in later stages of "cholangiolitic" cirrhosis.[54-56]

Discussion

The development of each type of cirrhosis is an extremely complicated four-dimensional process in which the three dimensions of space and the fourth dimension of time must be considered. The material for this study, however, consists essentially of two-dimensional serial sections of fixed tissue, the dimension of time having been eliminated by the death of the patient, the dimension of depth having been lost in the microtome. From these two-dimensional shadows and by the application of analytic mechanical principles, the attempt was made to reconstruct the four-dimensional process.

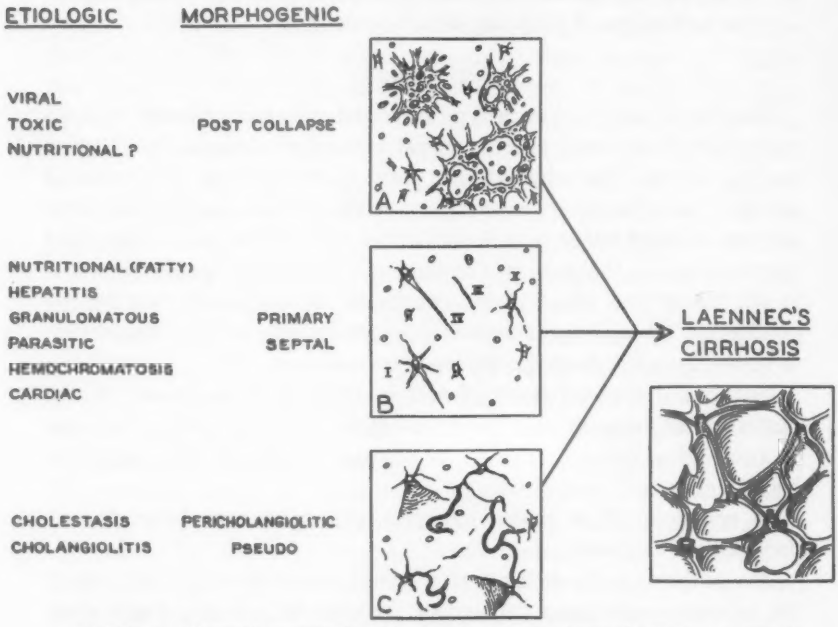
Cirrhosis is the end result of a series of different pathways. In all pathways, absolute or relative (as a result of condensation), increase of connective tissue is almost invariably associated with injury to hepatic cells.

In principle, three major morphogenetic pathways (Text-fig. 5) may initiate cirrhosis:

One pathway is by collapse after massive or submassive necrosis of the lobular parenchyma. Although the scar following collapse does not represent cirrhosis, septa resulting from fissures and periportal inflammation, as well as the formation of nodules of variable sizes and shapes in the surrounding tissue cause cirrhotic transformation, for which the term post-collapse cirrhosis may be justified. Typically, the lesion is not uniform in different parts of the liver and many portal triads and central canals may be entirely uninvolved either in larger nodules or in the lobular parenchyma. Although viral infections are probably the most important cause, intoxications cannot be excluded and, at least in the Tropics, malnutrition may be causative.

The second pathway is by the primary formation of septa: (1) from the portal triad; (2) from the central canal; (3) within the lobular parenchyma; or, (4) within fissures owing to uneven expansion of hepatic territories. Focal necrosis, fatty metamorphosis, and irregular regeneration are among the stimuli for this septum formation, which has a tendency to be uniform throughout and to involve all lobules.

Since primary septum formation in these instances causes the subdivision of the lobule and the formation of regenerative nodules, the term primary septal cirrhosis could be proposed for a lesion which may have several causes. Most important is probably malnutrition (which causes fatty metamorphosis). However, primary septal cirrhosis may also be due to hepatitis of various causes, granulomatous diseases, hemochromatosis, parasitic infestation, and even passive congestion.



Text-fig. 5. Schematic presentation of etiologic and morphogenetic factors, as well as of the pathways for the development of cirrhosis.

The third pathway starts with the accumulation of fibrous connective tissue around perilobular and intralobular ductules (pericholangiolitis) to produce a cylindriform network traversing the lobule but not dissecting it; therefore, the term cirrhosis does not apply to this stage, for which the term pericholangiolitic pseudo-cirrhosis could be used. In later stages, however, septum formation ensues with subdivision of the lobule, and it then becomes difficult to recognize the morphogenesis of the lesion which may be due to intrahepatic changes associated with cholestasis or, though rarely, to extrahepatic mechanical biliary obstruction.

It appears that eventually septum formation takes place in all three forms and a common terminal pathway results, for which the term Laennec's cirrhosis is proposed in analogy to the term Bright's disease applied to a similar stage in renal diseases.[30] The initial pathway may sometimes be recognized by large scars and large nodules in cases following massive necrosis and collapse[1,5]; but there are many cases of similar etiology in which small nodules of a diffuse septal cirrhosis are in the foreground.[19,21]

It should be stressed that the terms proposed refer to morphogenetic pathways and not to etiologic entities. The same liver may show indications of two pathways; for instance, in the diffuse septal cirrhosis due to alcoholic or tropical malnutrition, large scars resulting from primary and secondary collapse may be complicating features. The terms, therefore, are only partly comparable to the designations in general use. With this reservation, *post-collapse cirrhosis* corresponds to what is often called toxic,[1] or post-necrotic cirrhosis,[5] chronic liver atrophy,[20] or coarse nodular hyperplasia. *Primary septal cirrhosis* refers to lesions sometimes designated as portal or Laennec's cirrhosis, whereas *pericholangiolitic pseudocirrhosis* corresponds to some forms of biliary or Hanot's cirrhosis.[5,56]

For the basic clinical manifestations of cirrhosis, two morphologic features, common to all types, can be made to account; namely, the regenerative nodule and the anastomoses between portal and hepatic veins. In the regenerative nodule the liver cell plates have rearranged themselves. The direction of the liver cell plates in general reflects the direction of the blood flow,[49,39] and the altered flow in the nodule results in a tendency of the plates to become arranged concentrically to the new center of the nodule and independent of the center of the original lobule. Regeneration within the nodule is the result of an attempt to replace liver tissue near the interruption of the plate on the border of the nodules; but it is also the result of humoral effects caused by the reduction of liver tissue in general. This distant effect can be compared to the regeneration occurring in the intact liver of one of a pair of parabiotic rats when a partial hepatectomy has been performed on the other rat.[57] This regeneration leads to compression of the hepatic veins[9,11] which represents an important cause of portal hypertension in cirrhosis,[11] probably mechanically more important than excess of arterial blood supply to the cirrhotic septa.[22] The smaller the regenerative nodules, the more effective is their compression of the hepatic veins; therefore, the degree of portal hypertension is greater in the fine nodular form than in the coarse nodular form.[21]

The porto-hepatic venous anastomoses develop either in collapsed areas where sinuses persist or in the septa that connect the portal with the central field. In the septa the vessels usually form a dense network in which hepatic arterial branches participate,[22] as seen in injection preparations.[9,22] Through the anastomoses, blood is shunted from the portal to the hepatic vein, by-passing the lobular or nodular parenchyma and putting the parenchyma at a circulatory disadvantage. These shunts do not relieve portal hypertension because the compression of the hepatic veins previously described occurs closer to the heart than do these anastomoses. The anastomoses are considered responsible for nutritional disturbances and anoxia of the lobular parenchyma in cirrhosis; anastomoses likewise account for some of the instances of hypoxic (secondary) necrosis seen frequently in the center of the lobules and nodules. The shunts maintain injury to the cirrhotic parenchyma which persists even when the original or predisposing cause of cirrhosis, such as fatty metamorphosis or virus infection, has long disappeared. The central necrosis of lobules and nodules may become extensive and even submassive. Some cells of such a necrotic nodule or lobule may survive and become trapped in the connective tissue, either as isolated cells or in groups. They may eventually grow into new nodules. These nodules may become necrotic again in a never-ending cycle, characteristic of cirrhosis.

Summary

The attempt has been made to demonstrate the pathways of the architectural changes of the liver in various types of cirrhosis and to explain the mechanical processes associated with them.

1. One pathway results from the sequelae of massive and submassive necrosis of the lobular parenchyma with subsequent collapse, leading to post-collapse cirrhosis. The vessels and ducts are approximated and the angles of branching become acute without disturbance of the basic arrangement of interdigitation of the portal and hepatic canals. In the surrounding liver tissue a pattern of traction is created by the collapse of the necrotic area, and fissures arise in which connective tissue septa develop. Lobular fragments in submassive necrosis become nodules which either represent part of a lobule or may be composed of multiple lobules.

2. The second pathway leads to primary septal cirrhosis through the formation of septa by aggregation of collagenous membranes. The septa have formed either in the lobular parenchyma as reinforcement of the argentaffin fiber network or in stress fissures separating hepatic

territories of uneven expansion caused by regeneration, necrosis, and irregular fatty metamorphosis. Septa may originate from either portal or central canals or develop within the parenchyma. They may cut out nodules of various sizes from the lobule, especially if they connect central and portal canals. In the nodules, rearrangement of the liver cell plates, formation of a new efferent vein, and intense regeneration develop. The intense regeneration is reflected in plates several cells thick and in the formation of ductules.

In fatty metamorphosis septation may be the result of several processes: (a) collapse of fatty cysts; (b) periportal and intralobular necrosis and inflammation; and (c) membrane formation in stress fissures caused by uneven expansion of hepatic territories from irregular fat deposition, regeneration, and necrosis. Since processes (b) and (c) predominate in the human liver, cirrhosis from fatty metamorphosis does not result from fat deposition directly, but from increased susceptibility of the fatty liver to necrotizing or inflammatory processes and subsequent regeneration.

3. In pericholangiolitis, fibrous connective tissue proliferation in the adventitia of intralobular ductules creates irregularly curved cylindriform trabeculae. Even if this connective tissue formation becomes extensive (pericholangiolitic pseudo-cirrhosis), the lobule is not subdivided until late when septa form because of accompanying processes, and cirrhosis develops.

4. All three pathways—collapse, septation, and formation of cylindriform trabeculae—may terminate in a common pathway with extensive development of septa and nodules, for which the term Laennec's cirrhosis is proposed.

5. One feature common to any cirrhosis is nodule formation (a) from lobular fragments after collapse, (b) from subdivisions of the lobule by septa, or (c) from regeneration of trapped cells within the septa. The regenerating nodules compress the hepatic veins, thus accounting for portal hypertension. A second feature is porto-hepatic venous anastomoses by inclusion of sinusoids in developing septa. These shunt blood past the parenchyma and thus produce unfavorable circulatory conditions for it. Central necrosis ensues, setting in motion a cycle of degeneration and regeneration, characteristic of cirrhosis and independent of the persistence of its original cause.

REFERENCES

1. Mallory, F. B. Cirrhosis of the liver. *New England J. Med.*, 1932, 206, 1231–1239.
2. Moon, V. H. Histogenesis of atrophic cirrhosis. *Arch. Path.*, 1932, 13, 691–706.

3. Eppinger, H. Die Leberkrankheiten. Allgemeine und spezielle Pathologie und Therapie der Leber. Julius Springer, Vienna, 1937, 801 pp.
4. Hart, J. F., and Lisa, J. R. Histogenesis of Laennec's cirrhosis. *New York State J. Med.*, 1937, 37, 1619–1626.
5. Karsner, H. T. Morphology and pathogenesis of hepatic cirrhosis. *Am. J. Clin. Path.*, 1943, 13, 569–606.
6. Himsworth, H. P. Lectures on the Liver and Its Diseases: Comprising the Lowell Lectures Delivered at Boston, Massachusetts, in March, 1947. Harvard University Press, Cambridge, 1947, 204 pp.
7. Elias, H. A re-examination of the structure of the mammalian liver. II. The hepatic lobule and its relation to the vascular and biliary systems. *Am. J. Anat.*, 1949, 85, 379–456.
8. Elias, H., and Spanier, E. H. Structure of the collagenous tissue in the cirrhotic liver, a contribution to the geometry of sectioning. *Ztschr. f. wissensch. Mikr.*, 1953, 61, 213–221.
9. Popper, H.; Elias, H., and Petty, D. E. Vascular pattern of the cirrhotic liver. *Am. J. Clin. Path.*, 1952, 22, 717–729.
10. Rössle, R. Entzündungen der Leber. In: Henke, F., and Lubarsch, O. Handbuch der speziellen pathologischen Anatomie und Histologie. Julius Springer, Berlin, 1930, 5, 243–505.
11. Kelty, R. H.; Baggenstoss, A. H., and Butt, H. R. The relation of the regenerated liver nodule to the vascular bed in cirrhosis. *Gastroenterology*, 1950, 15, 285–295.
12. Elias, H. The liver cord concept after one hundred years. *Science*, 1949, 110, 470–472.
13. Randall, J. T.; Fraser, R. D. B.; Jackson, S.; Martin, A. V. W., and North, A. C. T. Aspects of collagen structure. *Nature, London*, 1952, 169, 1029–1033.
14. Glegg, R. E.; Eidinger, D., and Leblond, C. P. Some carbohydrate components of reticular fibers. *Science*, 1953, 118, 614–616.
15. Elias, H., and Popper, H. Histochemical factors in the formation of hepatic cirrhosis. (In preparation.)
16. Lucké, B., and Mallory, T. The fulminant form of epidemic hepatitis. *Am. J. Path.*, 1946, 22, 867–945.
17. Popper, H., and Franklin, M. Viral versus toxic hepatic necrosis. *Arch. Path.*, 1948, 46, 338–376.
18. Elias, H., and Pauly, J. E. Strukturelemente in menschlichen Nebennierenrinden. *Anat. Anz.*, 1953, 100, 134–147.
19. Werthemann, A., and Bodoky, G. Die pathologische Anatomie der gehäuften Leberdystrophiefälle von Basel aus dem Jahre 1946. *Schweiz. Ztschr. f. Path. u. Bakt.*, 1947, suppl., 10, 176–201.
20. Bjørneboe, M., and Raaschou, F. The pathology of subchronic atrophy of the liver. *Acta med. Scandinav.*, 1949, suppl. 234, 41–62.
21. Baggenstoss, A. H., and Stauffer, M. H. Post-hepatitic and alcoholic cirrhosis: clinicopathologic study of 43 cases of each. *Gastroenterology*, 1952, 22, 157–180.
22. McIndoe, A. H. Vascular lesions of portal cirrhosis. *Arch. Path.*, 1928, 5, 23–42.
23. Elias, H., and Petty, D. Gross anatomy of the blood vessels and ducts within the human liver. *Am. J. Anat.*, 1952, 90, 59–111.

24. Elias, H.; Bond, E., and Lazarowitz, A. The "normal" liver of the pig; is it an example of purely portal (and therefore subclinical) cirrhosis? A preliminary report. *Am. J. Vet. Research*, 1954, **15**, 60–66.

25. Thaler, H. Über die formale Pathogenese der posthepatitischen Lebercirrhose. *Beitr. z. path. Anat. u. z. allg. Path.*, 1952, **112**, 173–186.

26. Benz, E. J.; Baggenstoss, A. H., and Wollaeger, E. E. Atrophy of the left lobe of the liver. *A. M. A. Arch. Path.*, 1952, **53**, 315–330.

27. Moschcowitz, E. Laennec cirrhosis. Its histogenesis, with special reference to the role of angiogenesis. *Arch. Path.*, 1948, **45**, 187–215.

28. Ungar, H. Transformation of the hepatic vasculature of rats following protracted experimental poisoning with carbon tetrachloride. Its possible relation to the formation of urate calculi in the urinary tract. *Am. J. Path.*, 1951, **27**, 871–883.

29. Braus, H. Anatomie des Menschen. Julius Springer, Berlin, 1924, **4**, p. 317.

30. Popper, H. Liver disease—morphologic considerations. *Am. J. Med.*, 1954, **16**, 98–117.

31. Hartroft, W. S., and Ridout, J. H. Pathogenesis of the cirrhosis produced by choline deficiency. Escape of lipid from fatty hepatic cysts into the biliary and vascular systems. *Am. J. Path.*, 1951, **27**, 951–989.

32. Horstmann, E. Entwicklung und Entwicklungsbedingungen des intrahepatischen Gallengangsystems. *Arch. f. Entwcklngsmechn. d. Organ.*, 1939, **139**, 363–392.

33. Hess, O. Über die bei der akuten gelben Leberatrophie auftretenden Regenerationsprozesse. *Beitr. z. path. Anat. u. z. allg. Path.*, 1913, **56**, 22–62.

34. Eppinger, H.; Kaunitz, H., and Popper, H. Die seröse Entzündung. Julius Springer, Vienna, 1935, 298 pp.

35. Hill, K. R.; Rhoades, K.; Stafford, J. L., and Aub, R. Liver disease in Jamaican children (serous hepatosis). *West Indies M. J.*, 1951, **1**, 49–63.

36. Cameron, G. R., and Karunaratne, W. A. E. Carbon tetrachloride cirrhosis in relation to liver regeneration. *J. Path. & Bact.*, 1926, **42**, 1–21.

37. Popper, H. Unpublished observations.

38. Wallach, H. F., and Popper, H. Central necrosis of the liver. *Arch. Path.*, 1950, **49**, 33–42.

39. Elias, H., and Sokol, A. Dependence of the lobular architecture of the liver on the porto-hepatic blood pressure gradient. *Anat. Rec.*, 1953, **115**, 71–85.

40. Sherlock, S. The liver in heart failure; relation of anatomical, functional and circulatory changes. *Brit. Heart J.*, 1951, **13**, 273–293.

41. Moschcowitz, E. The morphology and pathogenesis of cardiac fibrosis of the liver. *Ann. Int. Med.*, 1952, **36**, 933–955.

42. Popper, H.; Szanto, P. B., and Elias, H. Transition of fatty liver into cirrhosis. *Gastroenterology*, 1955, **28**, 183–192.

43. Hartroft, W. S. Accumulation of fat in liver cells and in lipodiastaemata preceding experimental dietary cirrhosis. *Anat. Rec.*, 1950, **106**, 61–87.

44. Connor, C. L. Fatty infiltration of the liver and the development of cirrhosis in diabetes and chronic alcoholism. *Am. J. Path.*, 1938, **14**, 347–364.

45. Davies, J. N. P. Nutrition and Nutritional Diseases. In: Cutting, W. C. (ed.) Annual Review of Medicine. Annual Reviews, Inc., Stanford, Calif., 1952, **3**, 99–132.

46. Dible, J. H. Degeneration, necrosis, and fibrosis in the liver. *Brit. M. J.*, 1951, 1, 833–841.

47. György, P. Experimental hepatic injury. *Am. J. Digest. Dis.*, 1952, 19, 392–396.

48. Popper, H.; De la Huerga, J., and Koch-Weser, D. Hepatic injury due to conditioned sulfo amino acid deficiency. *Ann. New York Acad. Sc.*, 1954, 57, 936–947.

49. Pfuhl, W. Die Leber. In: von Möllendorff, W. Handbuch der mikroskopischen Anatomie des Menschen. Julius Springer, Berlin, 1932, 2, 235–425.

50. MacMahon, H. E., and Thannhauser, S. J. Congenital dysplasia of the interlobular bile ducts with extensive skin xanthomata: congenital acholangic biliary cirrhosis. *Gastroenterology*, 1952, 21, 488–506.

51. Eberth, C. J. Untersuchungen über die normale und pathologische Leber. *Virchows Arch. f. path. Anat.*, 1867, 39, 70–89.

52. Clara, M. Untersuchungen an der menschlichen Leber. I. Teil. Über den Übergang der Gallenkapillaren in die Gallengänge. *Ztschr. f. mikr.-anat. Forsch.*, 1930, 20, 584–607.

53. Hanger, F. M., Jr., and Gutman, A. B. Postarsphenamine jaundice apparently due to obstruction of intrahepatic biliary tract. *J. A. M. A.*, 1940, 115, 263–271.

54. Watson, C. J., and Hoffbauer, F. W. The problem of prolonged hepatitis with particular reference to the cholangiolitic type and to the development of cholangiolitic cirrhosis of the liver. *Ann. Int. Med.*, 1946, 25, 195–227.

55. MacMahon, H. E., and Thannhauser, S. J. Xanthomatous biliary cirrhosis (a clinical syndrome). *Ann. Int. Med.*, 1949, 30, 121–179.

56. Ahrens, E. H., Jr.; Payne, M. A.; Kunkel, H. G.; Eisenmenger, W. J., and Blondheim, S. H. Primary biliary cirrhosis. *Medicine*, 1950, 29, 299–364.

57. Bucher, N. L. R.; Scott, J. F., and Aub, J. C. Regeneration of the liver in parabiotic rats. *Cancer Research*, 1951, 11, 457–465.

LEGENDS FOR FIGURES

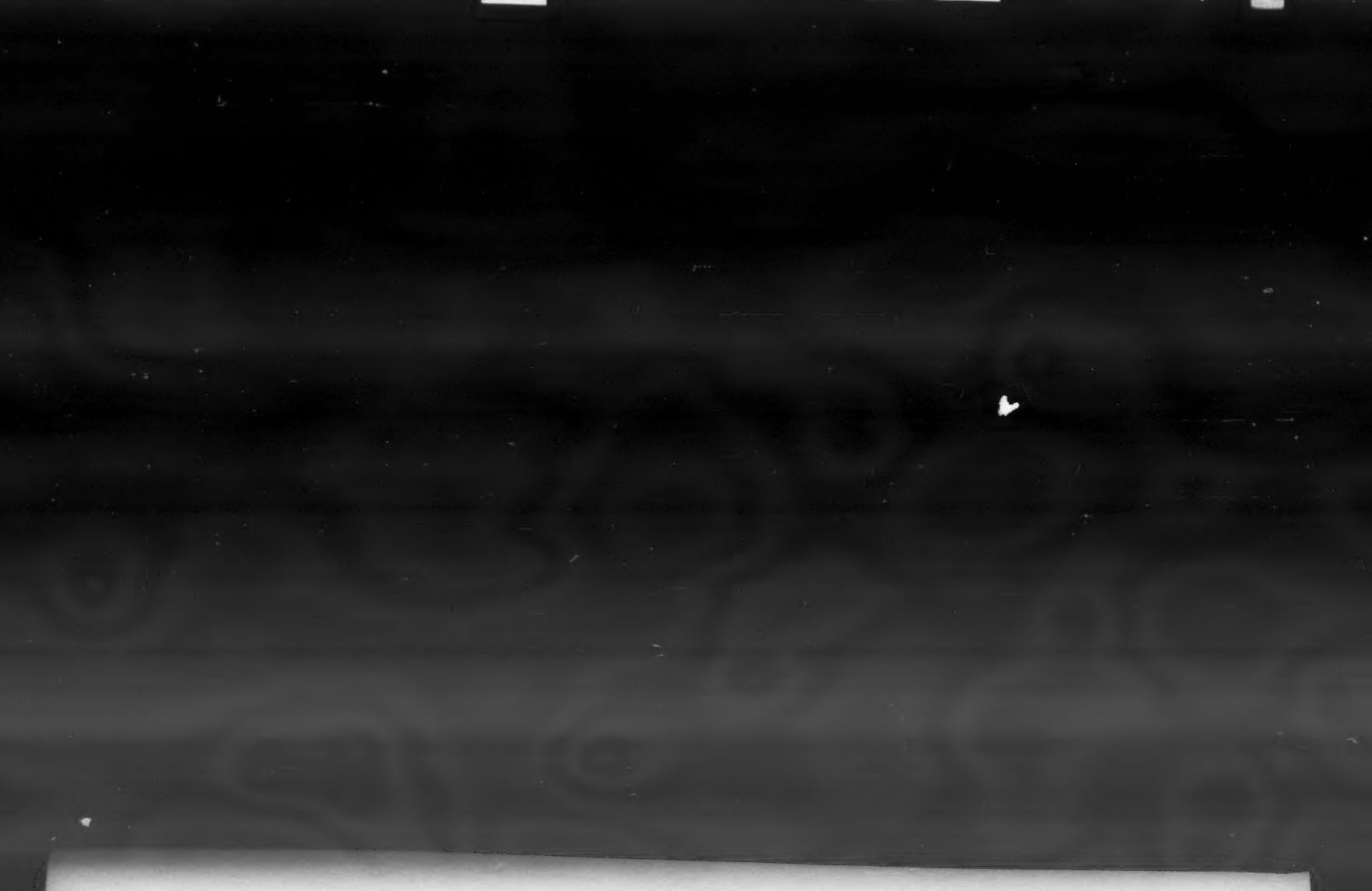
FIG. 1. Intact network of reticulum fibers during acute massive necrosis. Gomori's silver impregnation. × 450.

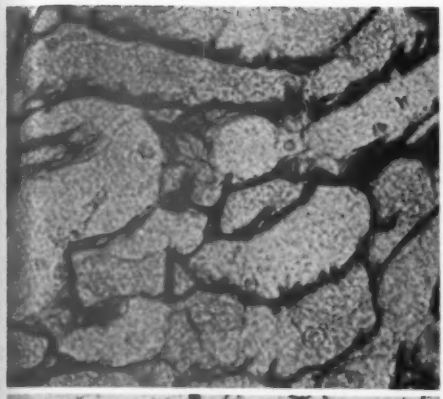
FIG. 2. Collapsed network of reticulum fibers after massive necrosis. Gomori's silver impregnation. × 450.

FIG. 3. Fuchsinophilic fibrillar material in area undergoing collapse. Section stained with van Gieson's stain and photographed on high contrast film. × 450.

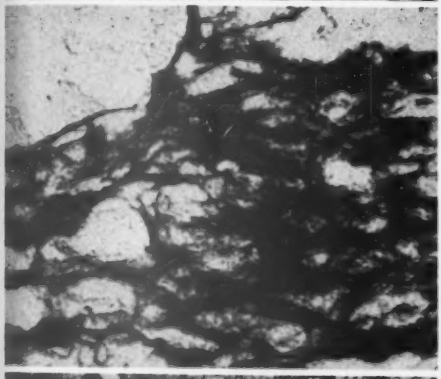
FIG. 4. Delicate collagenous membranes in collapsed area. Van Gieson's stain. × 450.

FIG. 5. Network of reticulum fibers in the same collapsed area as shown in Figure 4. Gomori's silver impregnation. × 450.



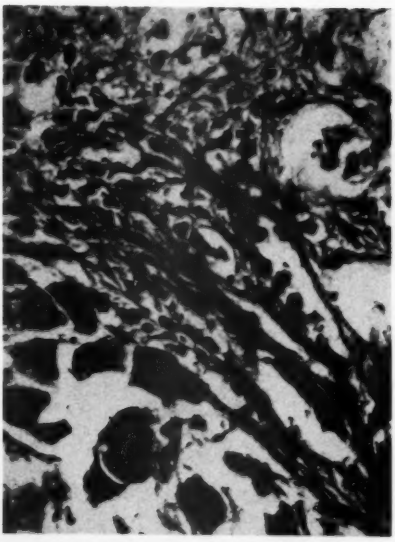


1

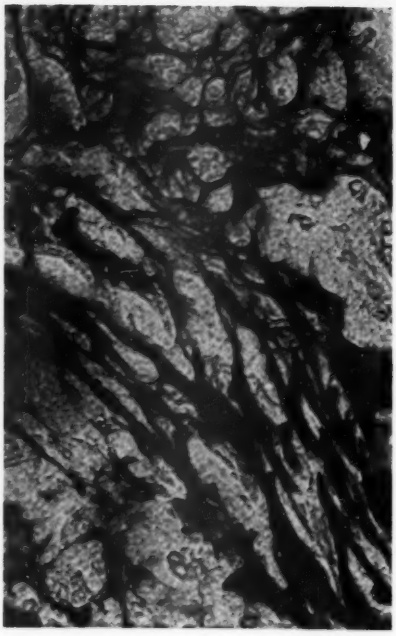


2

3



4

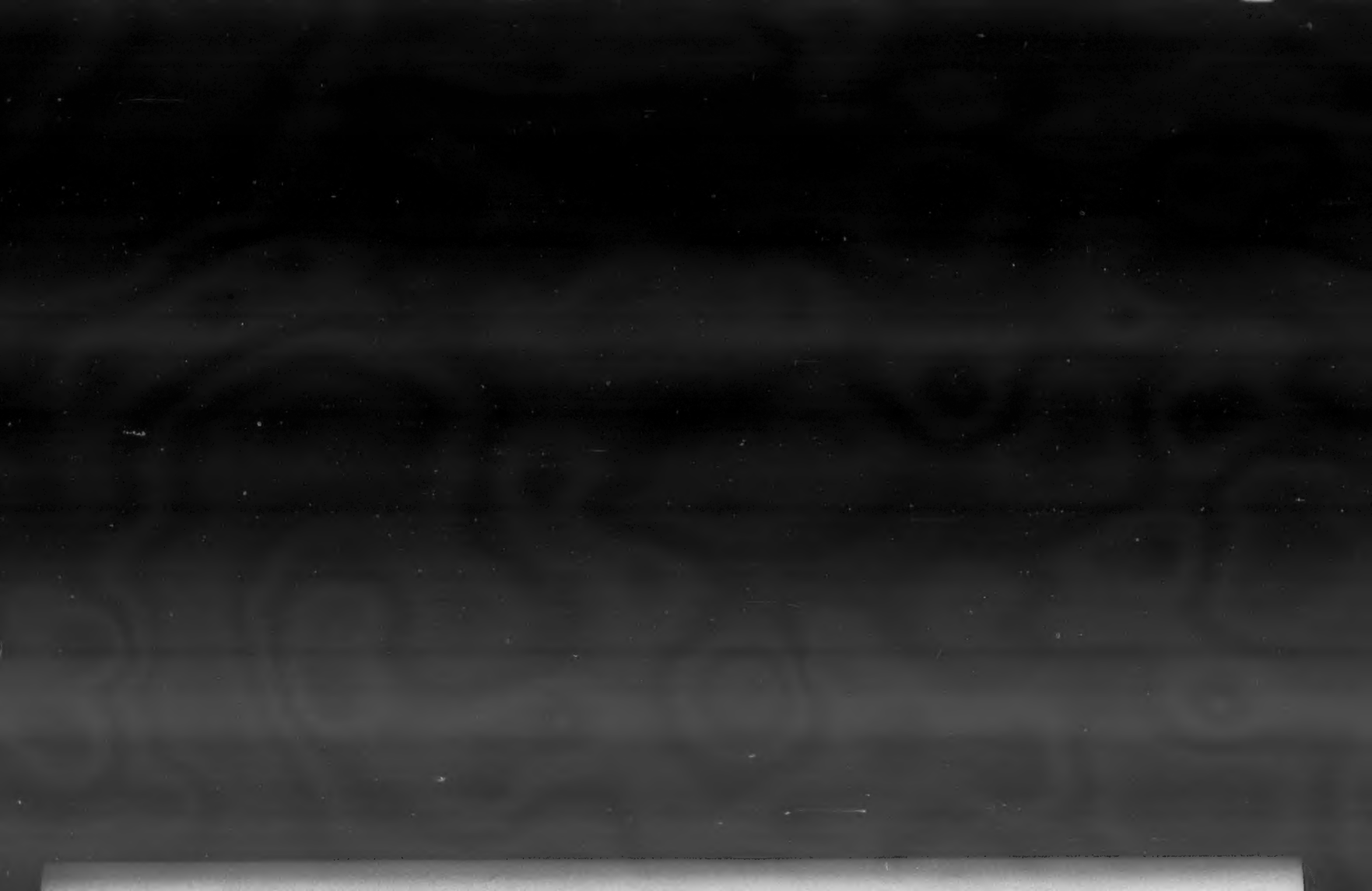


5

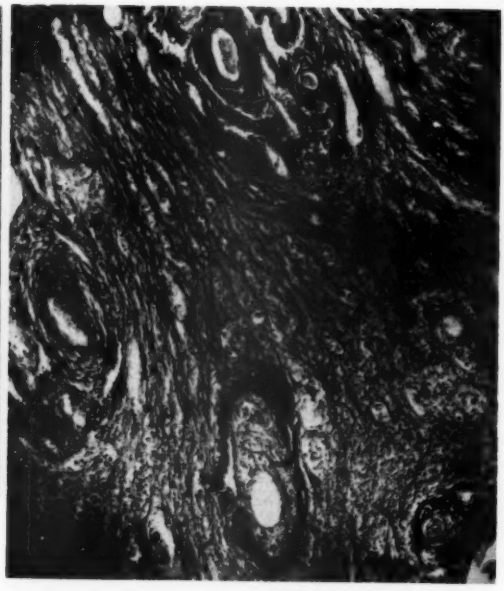
FIG. 6. Large scar resulting from collapse after massive necrosis (viral hepatitis). Approximation of portal and hepatic fields. Van Gieson's stain. × 61.

FIG. 7. Area of collapse after massive necrosis. Sharp difference between coarse fibers in portal and central canals, and delicate membranes in collapsed parenchyma. Van Gieson's stain. × 150.

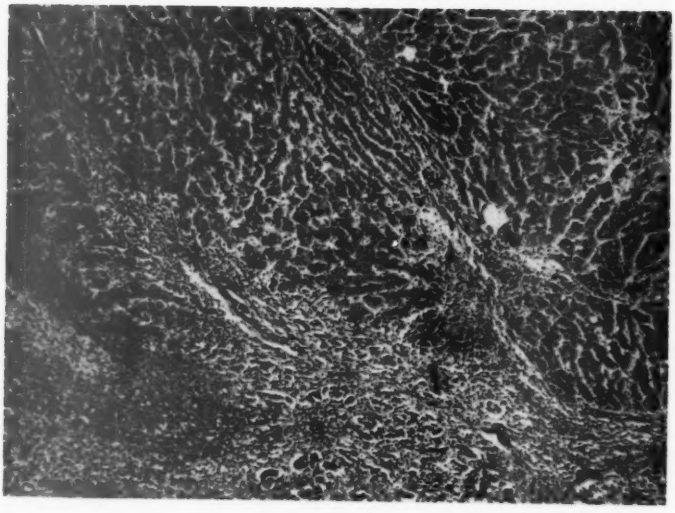
FIG. 8. Parenchyma bordering on massive necrotic area showing fissure due to traction resulting from adjacent collapse. These fissures have no relation to the lobular topography. Hematoxylin and eosin stain. × 60.



6



7



8

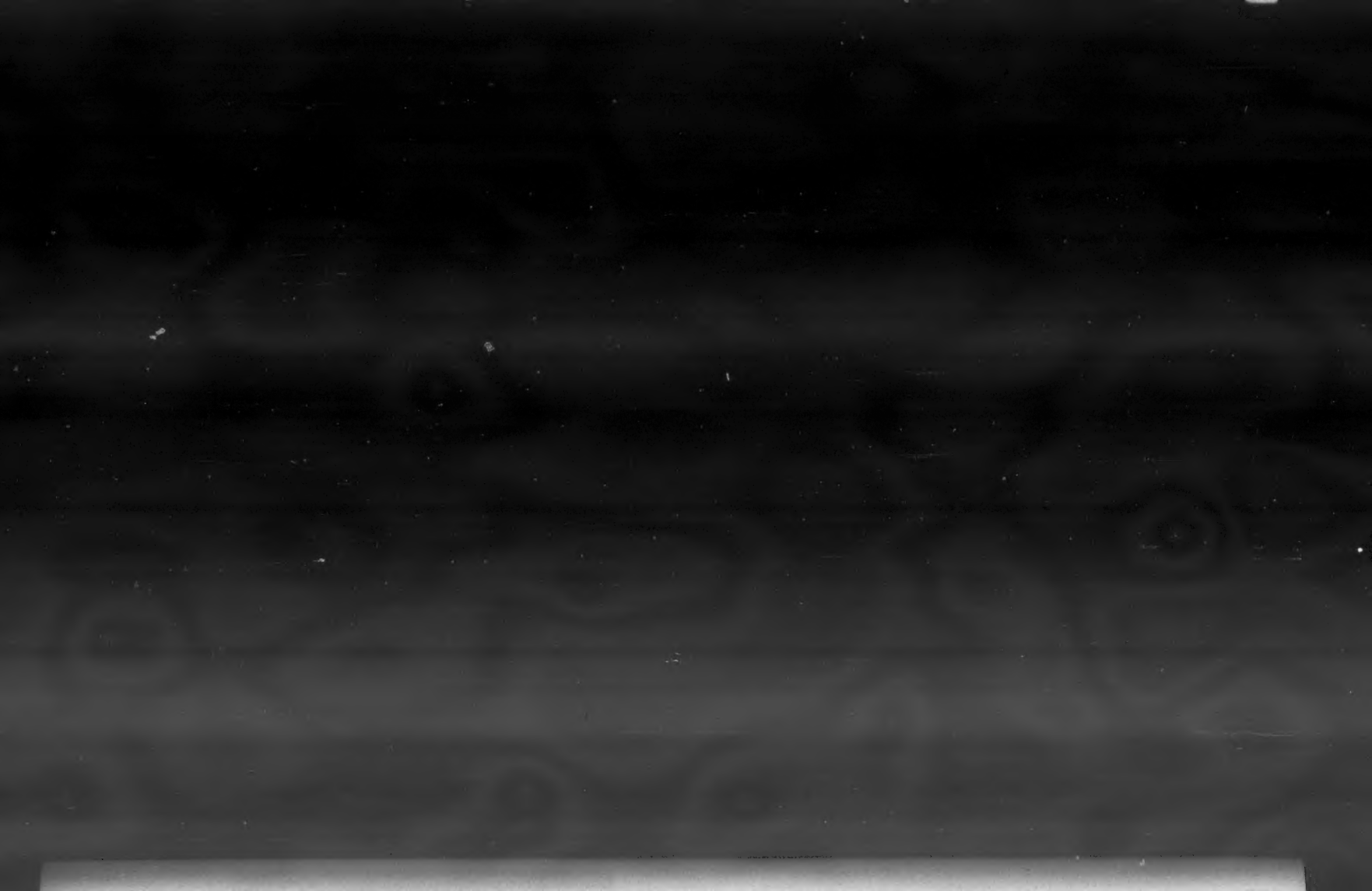
FIG. 9. Submassive collapse in viral hepatitis, with lobular fragments becoming nodular, and fissure due to stress. Hematoxylin and eosin stain. × 60.

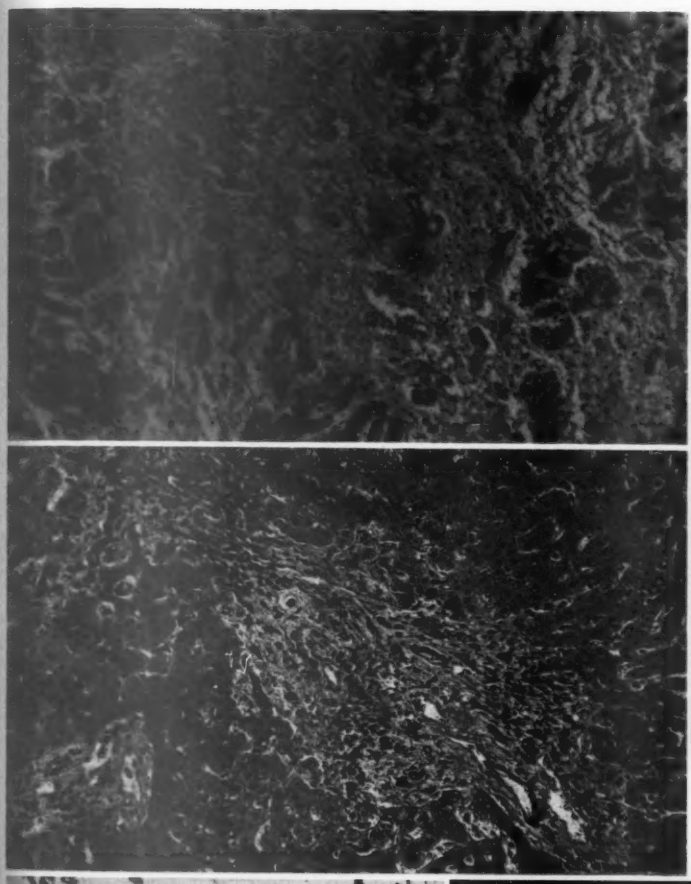
FIG. 10. Periportal inflammation and destruction of limiting plate. Hematoxylin and eosin stain. × 80.

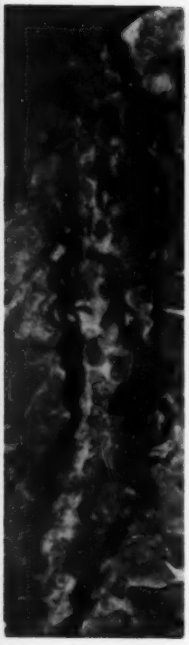
FIG. 11. Collagenous membranes radiating from portal canal, due to inflammation. Van Gieson's stain. × 110.

FIG. 12. Micromembranes aggregating to form membranes. Van Gieson's stain. × 550. (Reproduced by permission of the Josiah Macy, Jr. Foundation from *Transactions of the 11th Conference on Liver Injury*, 1952, page 188.)

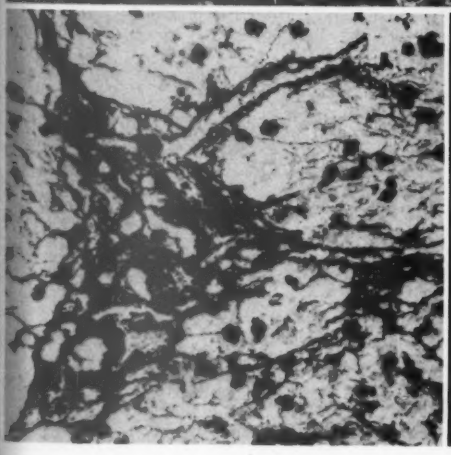
FIG. 13. Reconstruction of septa developing by aggregation of membranes and subdividing the lobule.

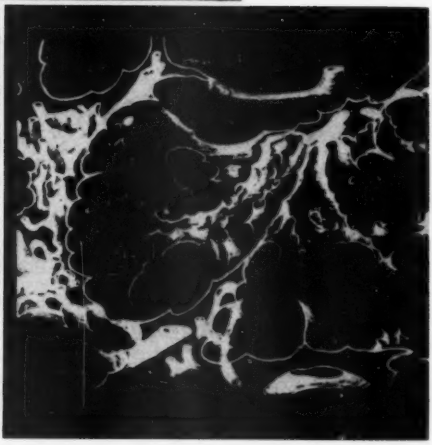






12





13

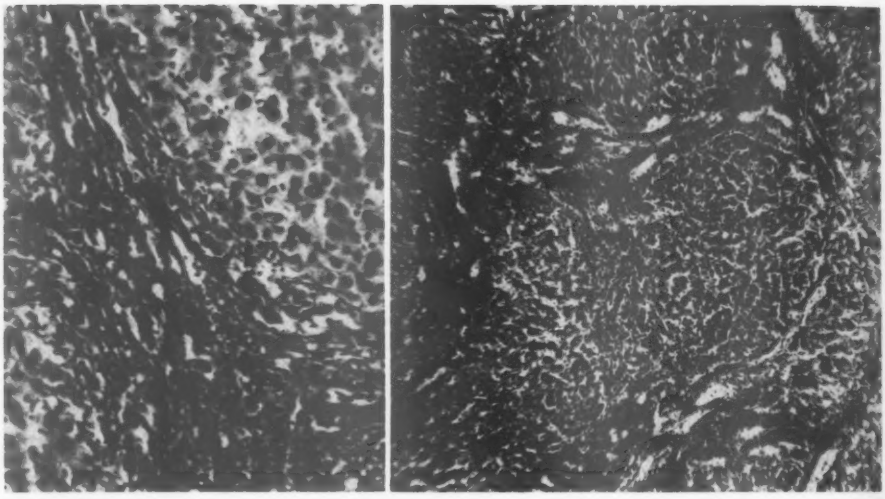
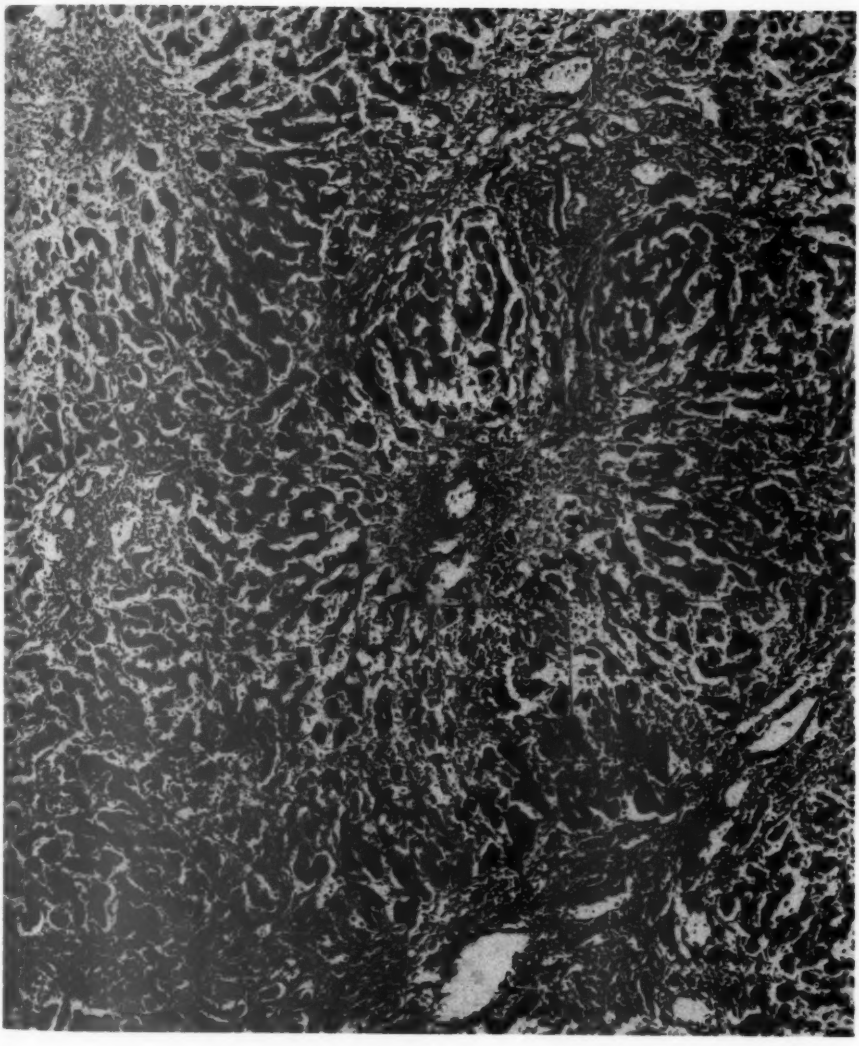


Fig. 14. Inclusion of sinusoids in developing septum. Van Gieson's stain. × 130.

Fig. 15. Connection of portal triads with each other and with central fields by septa. Van Gieson's stain. × 60.

Fig. 16. Subdivision of lobule by septa following central and periportal necrosis and membrane formation due to both chronic passive congestion and portal inflammation. A composite photomicrograph. Hematoxylin and eosin stain. × 80.



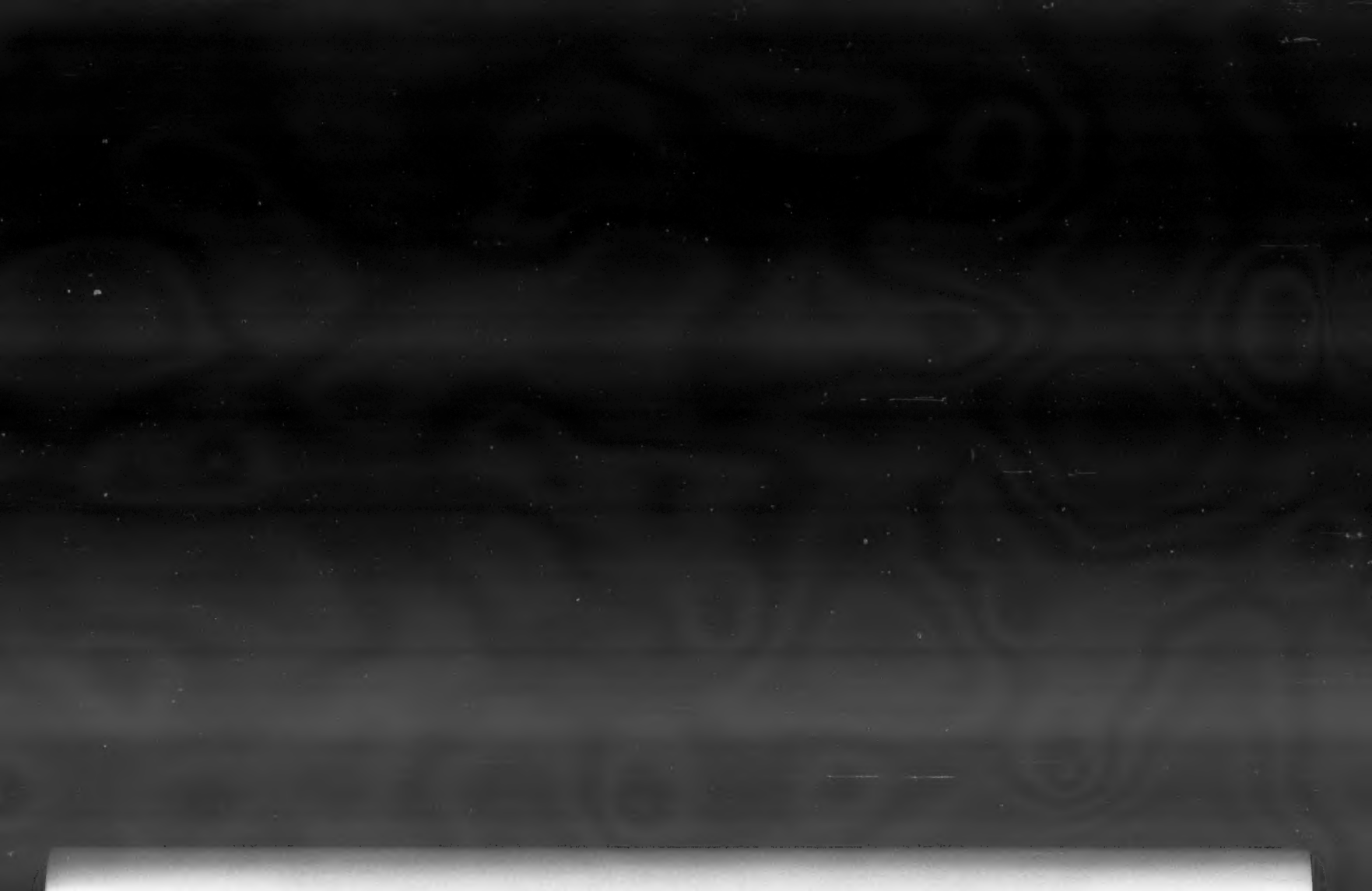
16

FIG. 17. Rearrangement of plates in lobular fragment (detail from Figure 16). Hematoxylin and eosin stain. × 280.

FIG. 18. Section from large regenerating nodule revealing liver cell plates several cells thick with amitotic division at the periphery, plates two cells thick in the intermediate zone, and plates one cell thick in the nodular center (below). Atrophic cells in surrounding septum. A composite photograph. Mallory's aniline blue stain. × 300.

FIG. 19. Same process as shown in Figure 18 in more rapidly growing nodule. Mallory's aniline blue stain. × 300.

FIG. 20. Central atrophy and necrosis in regenerated nodule. Hematoxylin and eosin stain. × 120.



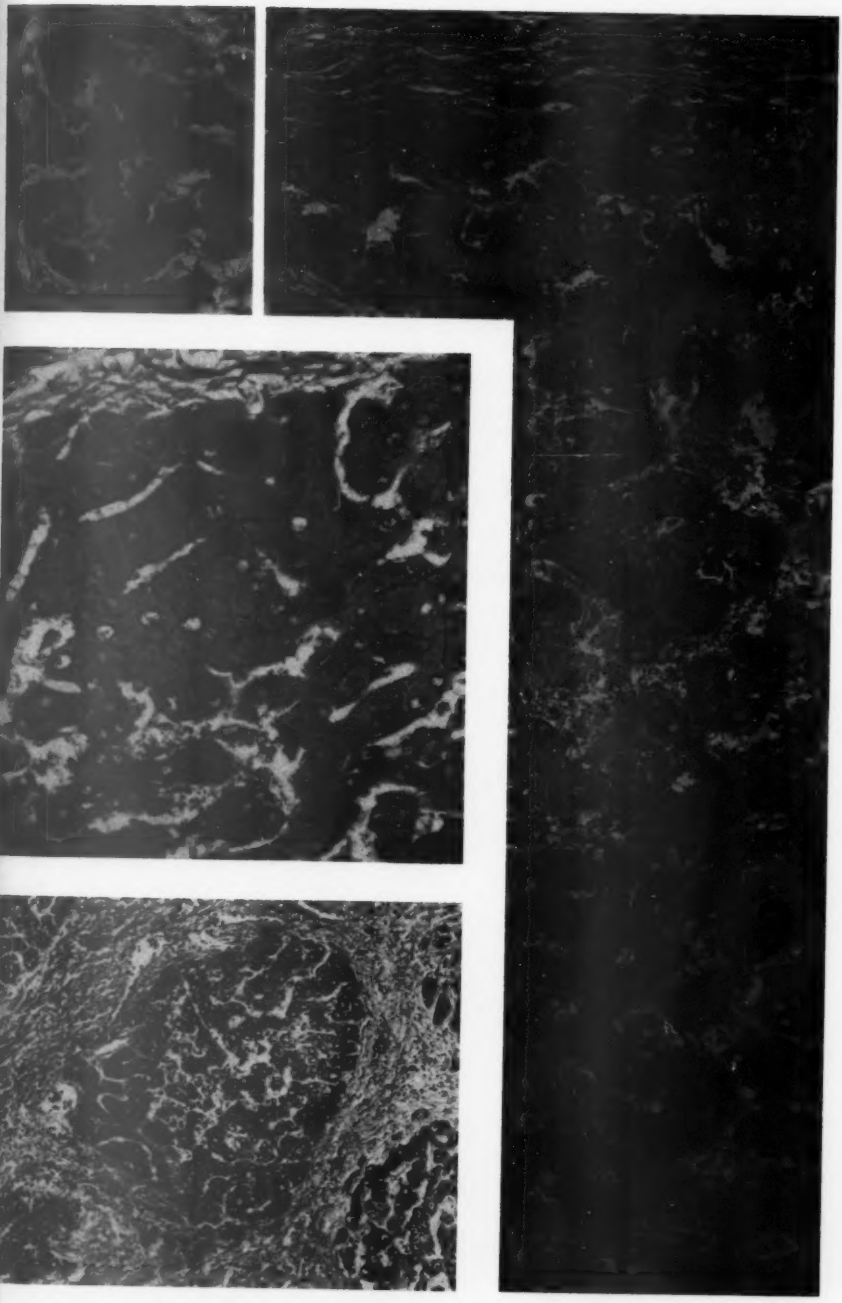


FIG. 21. Isolated atrophic liver cells and regenerating liver cells in small nodules in a cirrhotic septum. Hematoxylin and eosin stain. × 80.

FIG. 22. Connection of ductule with liver cell plates. Mallory's aniline blue stain. × 300.

FIG. 23. Fissure due to stress in a fatty liver, separating two parenchymal territories with different fat content and expansion. Hematoxylin and eosin stain. × 350.

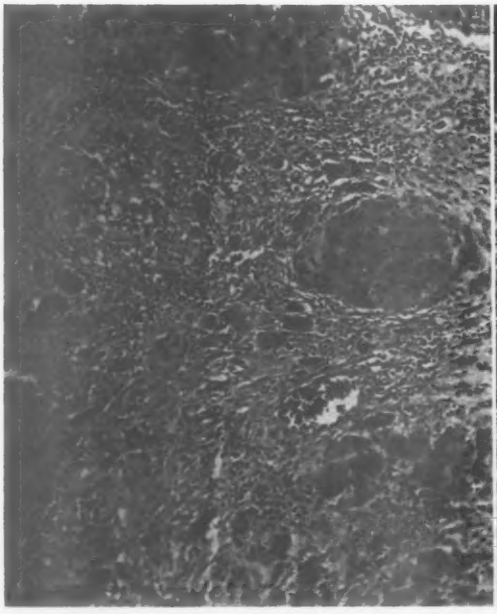
FIG. 24. Plates two cells thick with regeneration near fatty territories. Hematoxylin and eosin stain. × 230.

FIG. 25. Beginning deposition of collagenous membranes in fissure due to stress. Mallory's aniline blue stain. × 230.

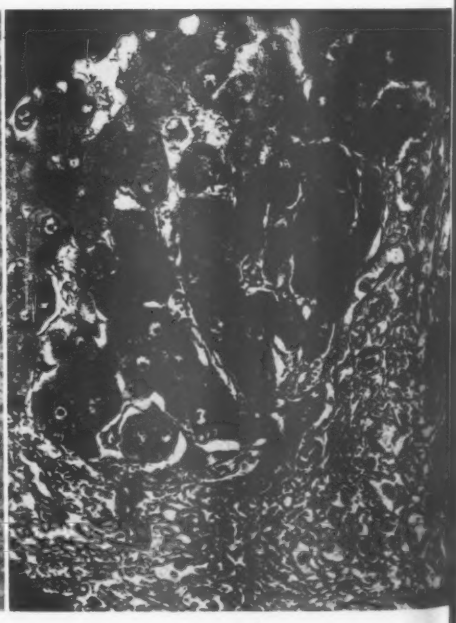
FIG. 26. Completed straight septum formed in stress fissure separating unevenly expanded lobular territories. Mallory's aniline blue stain. × 120.

FIG. 27. Fissure due to stress between regenerating and fatty hepatic territories. Hematoxylin and eosin stain. × 60.

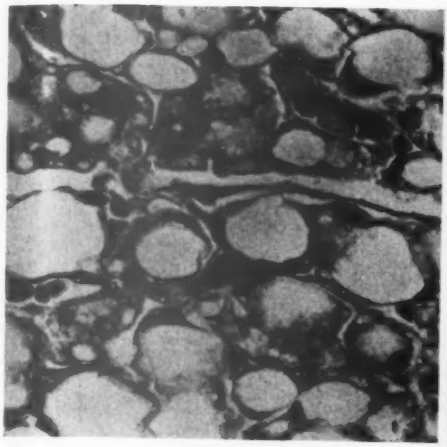
FIG. 28. Fatty cysts due to coalescence of fat droplets in several liver cells. Mallory's aniline blue stain. × 230.



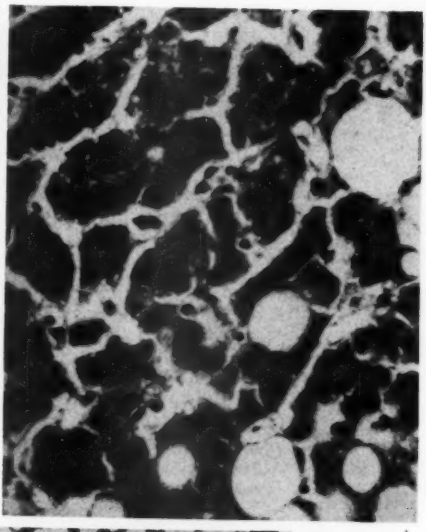
21



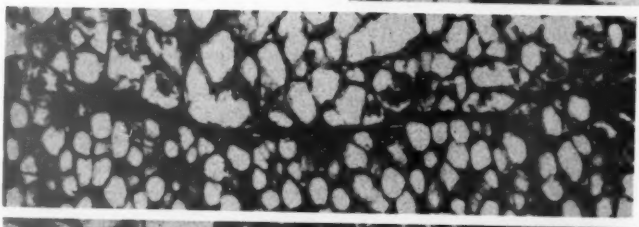
22



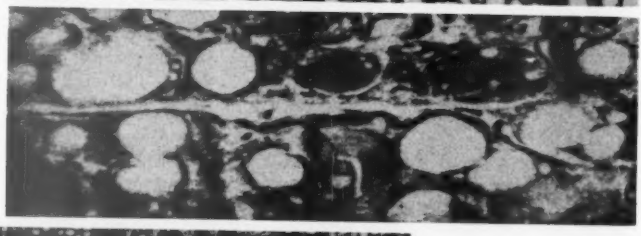
23



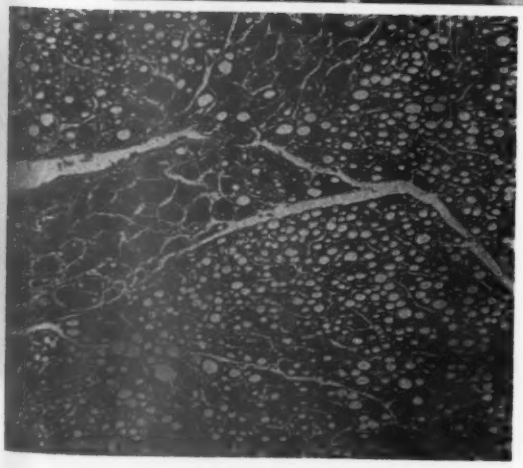
24



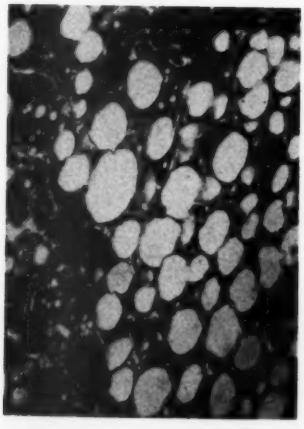
25



26



7



28

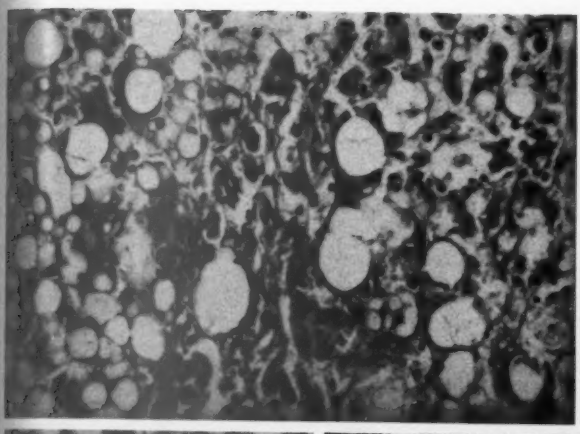
FIG. 29. Membranes developing near fatty cysts. Van Gieson's stain. × 230.

FIG. 30. Fibers developing near fatty cysts. Mallory's aniline blue stain. × 230.

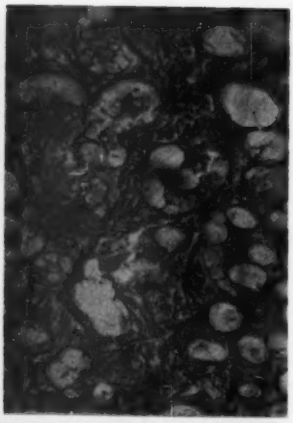
FIG. 31. Periportal radiating membranosis with septum formation following periportal inflammation with marked lipidosis. Mallory's aniline blue stain. × 60.

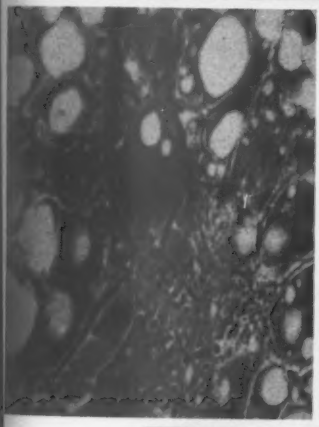
FIG. 32. Intralobular membrane formation following inflammation and necrosis associated with marked lipidosis. Mallory's aniline blue stain. × 230.

FIG. 33. Reconstruction of the connective tissue in advanced fatty cirrhosis, exhibiting the incomplete separation of the nodules. Drawn from a wax plate model by Nelson Brown.

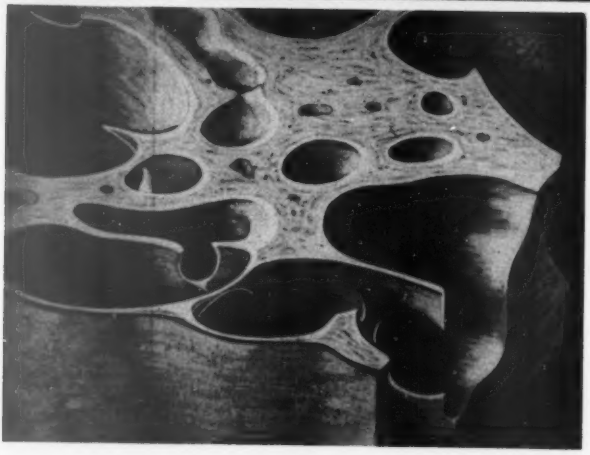


30





32



33

FIG. 34. Large intralobular bile ductule accompanied by an arteriole, both invested in a common adventitia. Hematoxylin and eosin stain. × 230.

FIG. 35. Intralobular cylindriform trabecula ensheathing ductules with pericholangiolitic inflammation. Of note is the elliptic shape of the section of the trabecula, which is different from a septum. Van Gieson's stain. × 60.

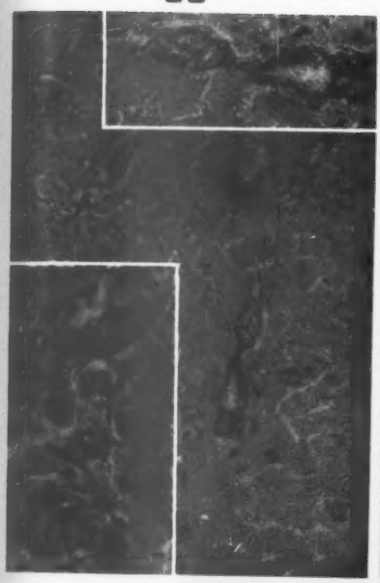
FIG. 36. Detail of Figure 24. Oblique section of cholangiolitic trabecula showing oblique and transverse sections of fibers. Van Gieson's stain. × 230.

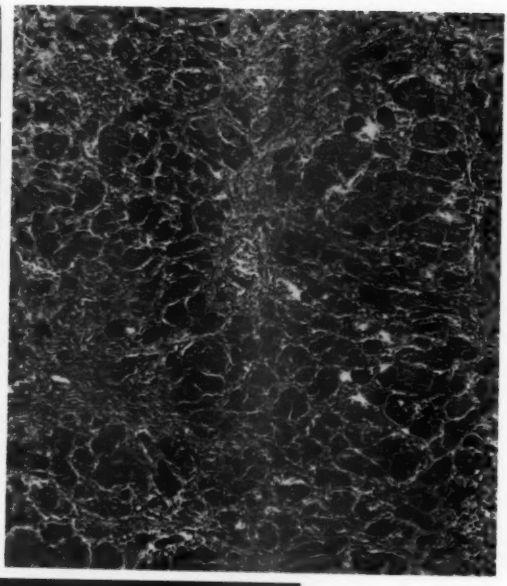
FIG. 37. Cholangiolitic cirrhosis with maintenance of lobular architecture, the parenchyma being traversed by trabeculae. Hematoxylin and eosin stain. × 60.

FIG. 38. Reconstruction of curved and cylindric trabeculae in cholangiolitic cirrhosis.

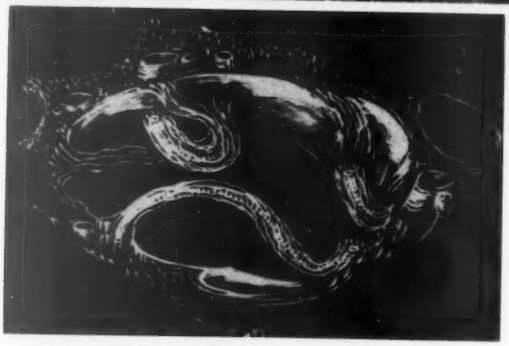
FIG. 39. Drawing showing the lobules traversed by increased and thickened cylindric trabeculae containing cholangioles. The lobular pattern is not disturbed.

36

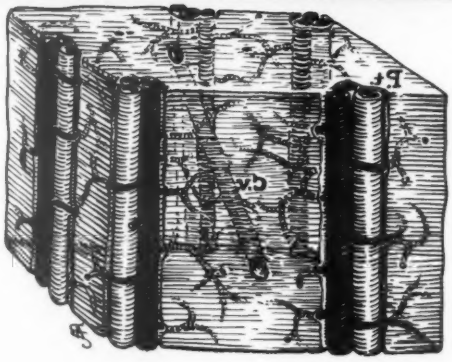




37



38



39

FOCAL MYOCYTOLYSIS OF THE HEART *

MONROE J. SCHLESINGER, M.D.,† and LEOPOLD REINER, M.D.
From the Pathology Laboratory, Beth Israel Hospital, and the Department of Pathology, Harvard Medical School, Boston, Mass.

In this communication we wish to focus attention on a miliary lesion of the myocardium not hitherto emphasized and commonly seen in coronary heart disease. We have characterized the lesion as focal myocytolysis. Others have described it under various designations such as acute miliary infarction,[1] focal necrotizing myocarditis without interstitial infiltration,[2] and sarcolytic myocardosis.[3]

Focal myocytolysis as it appears at the borders of cardiac infarcts was appreciated as early as 1904 by Smith,[4] but seemingly has since been forgotten. It has been described also in various noncoronary cardiac conditions.[1,2,5-9] Focal myocytolysis of the heart has been noted in several diseases not essentially cardiac.[3,10-12] It has been produced experimentally in a variety of animals.[13-16] In spite of its rather common occurrence, its morphologic and functional significance has not been appreciated generally.

In speed of evolution and histologic details, focal myocytolysis is intermediate between the reversible degenerations of the myocardium and the irreversible coagulation necrosis of an infarct. The lesion is closely similar to, and probably has been confused with, some forms of infarction. Hence, it is necessary first to delineate our concept of infarction in the heart.

General Characteristics of Cardiac Infarct

Cardiac infarct comprises three phases: necrosis, reactive exudation, and repair. By standard morphologic criteria, an infarct does not become demonstrable, grossly or histologically, for 12 to 24 hours after its *clinical* onset.

Necrosis involves not only the muscular syncytium but also the cardiac stroma. With the death of the muscle, its fibers at first increase, and later diminish, in diameter. The diminution is accompanied by smudginess, blurring of striations, increased eosinophilia, and karyolysis of the muscle nuclei. Necrosis of the stroma is shown by

* This study was supported by grants from the United States Public Health Service (Grant H-1121), the American Heart Association, and the Lebanon County Heart Association.

Received for publication, July 22, 1954.

† Died, January 20, 1955.

fragmentation and granularity of its reticular fibrils, blurring and altered staining qualities of its collagen, and karyolysis of its nuclei. Lysis of this necrotic tissue starts early in the periphery of the infarct and continues in a centripetal direction throughout the later phases. Acute exudative phenomena develop, with polymorphonuclear leukocytes as the most conspicuous single element. These cells are soon replaced by subacute and chronic inflammatory cells. Repair, in the form of granulation tissue, is discernible early and, with progressive fibrosis, becomes increasingly conspicuous. Healing, i.e., complete fibrous substitution, varies with the size of the infarct and, in the case of very large ones, requires many months.

Reactive exudation and repair are vital processes and thus can originate only from a viable stroma. In larger infarcts the supportive stromal structures of the heart always become necrotic. Therefore, reactive exudation and repair can manifest themselves only about the periphery of such infarcts as zonal and marginal phenomena. Repair proceeds, of necessity, in a centripetal direction.

Special Characteristics of Small and Miliary Infarcts

An important morphologic variation is introduced when the cardiac infarct is small. The basic phenomena of necrosis, lysis of necrotic tissue, reactive exudation and repair, as noted, remain identical, but they are completed in a shorter time. In small infarcts the early polymorphonuclear leukocytic infiltration may appear either zonally about the periphery or diffusely throughout the infarct. Polymorphonuclear leukocytes can advance from the viable marginal stroma only for a limited distance. Consequently, an infarct with a diameter of less than twice this distance will be diffusely infiltrated; one larger than that will not.

Apart from differences of size, smaller infarcts may differ in one other significant detail from larger ones inasmuch as their stroma does not necessarily die. If the stroma remains viable, such an infarct lacks the marginal zoning of reactive exudation and repair which are so characteristic of large infarcts. Instead, reactive exudation and repair are manifest *throughout* the small infarct. The necrotic muscular syncytium is the only element to be removed. Repair then proceeds largely by stromal collapse and stromal condensation and only slightly by fibroblastic proliferation. This detail of stromal survival of the miliary infarct sharply illustrates the differential sensitivity of stroma and muscle to the same degree of metabolic imbalance.

Characteristics of Focal Myocytolysis of the Heart

The lesion of focal myocytolysis, usually not larger than 1500 μ, closely resembles a miliary infarct without stromal necrosis, but differs in some essential respects. In this form of myolysis the muscle fibers disintegrate within a small and discrete territory. Their myofibrils seem simply to disappear (Figs. 1 and 2). The muscle nuclei of the affected fibers do not undergo rhexis, lysis, or pyknosis, but remain visible for some time. At most, there is some nucleoplasmic clumping (Figs. 1 and 2). The sarcolemma is preserved but it collapses and becomes increasingly difficult to distinguish from the intact stroma (Figs. 3 and 4). It looks as though the muscle fibrils had lost their syncytial integrity and had melted out or "fallen out" of their stromal envelopes. It is this appearance on a microscopic slide which suggested the colloquialism "falling-out necrosis" in use in our laboratory. No reactive exudation of polymorphonuclear leukocytes is elicited. At most, an occasional lymphocyte is present (Figs. 11 and 12). The center of the focus cleanses itself by progressive myolysis. At the periphery other muscle fibers disintegrate similarly (Figs. 11 and 12). This feature gives the process, in contrast to the *centripetal* nature of smaller and larger infarcts, a *centrifugal* character. This peripheral extension also is responsible for the fuzzy borders of focal myocytolysis (Figs. 3 and 4). Eventually, all that remains is a small focus of empty but intact cardiac stroma, in the meshes of which there are mononuclear cells, more or less laden with a finely granular, light-brown pigment (Figs. 13 and 14).

Focal myocytolysis and miliary infarcts without stromal necrosis both terminate in a fibrous tissue scar (Figs. 13 and 14). In both, this scarring is brought about largely by collapse and condensation of the preserved stroma rather than by proliferation of fibroblasts and elaboration of collagen. For neither process has the chronologic interval from onset to scarring been established. However, focal myocytolysis appears to be a more leisurely and less explosive process than infarction.

We do not know what is contained, during life, inside the loose, stromal meshwork of this focal lesion. With some exceptions about the periphery (Figs. 6 and 8), this meshwork appears largely empty. The empty appearance may be an artifact of fixation. On the other hand, the spaces may have contained a fluid so low in protein that it remained unstained. The latter hypothesis would be consistent with the

non-inflammatory character of the process. It may also, in part, account for the absence of proliferative fibrosis.

The intracellular, light-brown pigment, which has been referred to, is non-ferrous. It resembles the perinuclear pigment of muscle cells. These pigment-laden cells may be either macrophages or the nucleated, non-fibrillary remnants of partially lysed muscle fibers with their polar pigment still in place. The latter interpretation is supported by the rare observation of cross-striated fibrils within or attached to these cells. The nuclei of these cells are not distinctive enough in size, shape, or chromatin configuration to make decision of this question possible. Both interpretations as to the nature of these cells are probably valid.

In essence, then, focal myocytolysis is conceived as a local loss of myocardial syncytium, the result of a metabolic imbalance which is insufficient in intensity or duration or both to cause stromal injury or to elicit any reactive exudation. Its morphologic characteristics are: (1) disappearance of myocardial syncytium in foci usually not exceeding 1500 μ in diameter (Figs. 11 and 12); (2) absence of reactive exudation; (3) survival of the original cardiac stroma; (4) mononuclear cells containing a light-brown pigment; (5) centrifugal spread; (6) scarring of the focus by stromal collapse. There is no regenerative activity of the myocardial syncytium.*

A lesion of active focal myocytolysis (Figs. 1 to 6) as just described consists of a hypomyocytic or amyocytic center with pigment-laden cells in a loose stroma. About this there is a narrow border zone of myolysis. This centrifugal myolysis rarely occurs simultaneously about the whole circumference of all active foci. In some segments of the border of the foci one or the other of the following stages may be present:

An *inactive* or *subsiding* stage of focal myocytolysis (Figs. 7 to 10), which is characterized by pigment-laden cells lying in a collapsed loose stroma. There is no active myolysis.

A *healing* stage of focal myocytolysis (Figs. 11 to 14), which consists of more compact stromal remains and contains few or no pigment-laden cells.

A *healed* lesion of focal myocytolysis (Figs. 13 and 14), which is a tiny fibrous tissue scar, indistinguishable from a healed miliary infarct.

* In experimental narrowing of the coronary artery in young suckling pigs we have encountered a lesion similar to focal myocytolysis of human hearts. In these young animals, however, regeneration of muscle fibers did take place, as indicated by occasional muscle giant cells.

At times, one may detect the pattern of the lesion incorporated in a larger territory of myocardial infarction. This is interpreted as a sudden deterioration of a borderline metabolic imbalance, resulting not only in secondary infarction of foci already embarrassed by focal myocytolysis but also in infarction of the adjacent, previously normal myocardium.

Localization of Focal Myocytolysis of the Heart

The sequence of marginal localization and centripetal progression of reactive exudation and repair about larger cardiac infarcts has been emphasized. These large infarcts, however, display also certain centrifugal manifestations which are not usually separated from the centripetal progression. These are, first, stellate foci of myocardial disintegration which are seemingly discontinuous with the main infarct territory. Some of these are separate miliary infarcts with or without stromal necrosis but others are typical foci of myocytolysis. These lesions are situated preferentially about the external (pericardial) aspects of the infarct.

Another and uniquely centrifugal phenomenon is a discontinuous rim of myocytolysis which affects the viable myocardium of the very borders of large infarcts. This may be found long after the result of the main metabolic accident is well on its way to complete repair. It is not uncommon about the periphery of fully collagenized infarct scars. Its presence indicates that an unbalanced metabolic state may persist for a long time in such border territories.

However, myocytolysis is not confined to the borders of large infarcts. It also rims, more or less discontinuously, many miliary infarcts. Once these are transformed into scars, however, the two processes are indistinguishable.

Focal myocytolysis is also found in many hearts without infarcts, large or miliary. A favored location is the central portion of papillary muscles and trabeculae carneae of the left ventricle (Figs. 5 and 6). Fibrosis in these locations is a common finding in hearts which may or may not show diffuse fibrosis elsewhere. After these centers have become fibrotic, it is impossible to determine whether such minute loss of muscle syncytium was due to miliary infarction or to focal myocytolysis. It appears significant, however, that at the time of death centropapillary and centrotrabecular myocytolysis is much more common in these hearts than are fresh or healing miliary infarcts.

As shown by our injection studies, these centers are situated at the ends of the pure coronary vascular circulation. The metabolic exchange

of the layer of myocardium, which is situated between these centers and the endocardium proper, proceeds in part by trans-endocardial diffusion. The latter source can ordinarily nourish the subendocardial zone for a depth not exceeding the width of about a half a dozen muscle fibers. Even with large infarcts this zone frequently escapes necrosis. The width of this zone is comparable to that of the combined intima and inner third of the media of the adult aorta. This aortic zone has two similar avenues of metabolic exchange, that is, the vasa vasorum and trans-endothelial diffusion.[17]

Incidence of Focal Myocytolysis

In a detailed microscopic study of 571 hearts, an average of eleven sections were examined per heart, and each section was labelled as to its exact location; focal myocytolysis was found in 101 (17.7 per cent) hearts.* In Table I is indicated its incidence in relation to fresh

TABLE I

Incidence of Focal Myocytolysis in 571 Hearts. Association with Recent and Old Infarction

Focal myocytolysis	Recent infarcts, large and small	Fibrosis, including scarred infarcts	No infarcts, no fibrosis	Total
Present	51	45	5	101
Absent	29	233	208	470
Totals	80	278	213	571

infarcts and to fibrosis of the myocardium. The lesion was present in almost two thirds (51/80) of the hearts with recent infarcts. An almost equal number (50) of the hearts without fresh infarcts showed the lesion in question. Nearly all of these (45/50) also exhibited fibrosis of the myocardium. The 5 hearts which were free of both fibrosis and infarction were especially interesting. In them early lesions could be studied to great advantage. None of these 5 hearts had significant atherosclerosis of the coronary arteries and only one was hypertrophied. Associated clinical conditions were uremia (three) and malignant tumors (three).

Degree of Focal Myocytolysis

In the 101 hearts which displayed focal myocytolysis, a rough estimate was made of the extent of the lesion. Table II presents these

* This series of 571 hearts was studied by a method of injection and dissection previously described.[18] A correlation between the condition of the coronary arteries and the myocardial histopathology will be published subsequently.

estimates in relation to the simultaneous absence or presence of infarcts. There were 39 hearts with 1 per cent or less of myocytolysis in the total volume of myocardium examined. Such small amounts were three times as common in hearts without infarcts as in those with infarcts. There were 10 hearts with between 10 and 38 per cent of focal myocytolysis. In this group the lesion was associated with in-

TABLE II

Degree of Focal Myocytolysis in 101 Hearts

	Focal myocytolysis				
	Less than 1%	1.1-5.0%	5.1-10%	More than 10%	Total
No infarcts	29	18	1	2	50
Infarcts, large and small	10	17	16	8	51
Total	39	35	17	10	101

farcts in the great majority (8/10). The intermediate group which was losing cardiac muscle via myocytolysis at the rate of from 1.1 to 10 per cent, comprised a little more than one half (52) of the 101 hearts. In this group there were almost twice the number of hearts with infarction as without. The left ventricle was much more richly sampled in the selection of the sections than any other of the heart chambers. Focal myocytolysis is more common in the left ventricle than in the right. Hence, the degree of this lesion would be even greater had it been expressed in terms of the left ventricle alone rather than of the whole heart.

DISCUSSION

Aside from its association with coronary atherosclerosis, as has been emphasized, focal myocytolysis of the heart has been found as the predominant or incidental lesion in non-coronary diseases of man. It has been emphasized in intractable congestive failure and cardiomegaly of unknown etiology.[1,5-7] In some of these instances it has been ascribed to lues and to infections of viral or bacterial origin. It has been observed in Fiedler's myocarditis,[8] with coronary arterial embolism following subacute bacterial endocarditis,[9] scleroderma,[10] poliomyelitis,[11] uremia,[12] pregnancy and the puerperium,[5] and therapeutic insulin shock.[3]

Focal myocytolysis has been produced experimentally by oxygen deprivation in cats,[14] by adrenalin injections into rabbits,[15] by induction of hyperthyroidism, tachycardia, or both in rabbits,[16] and by diets free of vitamin B and deficient in proteins in albino rats.[13] The

lesions produced by hypopotassemia in rats[19] bear some similarity to focal myocytolysis.

The great variety of conditions in which it has been encountered suggests that the lesion constitutes the final morphologic pathway for many different etiologic agents, both natural and experimental. We conceive that the common denominator of these dissimilar agents, which produce identical morphologic changes, resides in the production of a metabolic imbalance in the heart. By this term we refer to abnormalities of tissue anabolism and catabolism such as may result from ischemia, anemia, hypotension, hypoglycemia, septicemia, toxemia, heart failure, cardiac dilatation, and nutritional disturbances.* In order to become manifest in routine microscopic sections, the metabolic imbalance will have to act with a great enough intensity and for a long enough time. The combination of these two factors may result in a degree of metabolic imbalance so slight as to cause only reversible lesions of the muscle fibers such as cloudy swelling, hydrops, or fatty infiltration. On the other hand, the degree may be so severe as to cause the irreversible necrosis of an infarct. These relationships are indicated in Table III, with the implication that the graded ana-

TABLE III

Schema of Regressive Cardiac Responses

	Degree of metabolic imbalance		
	Mild	Moderate	Severe
Lesion	Cloudy swelling Hydrops Fatty degeneration	I. Focal myocytolysis 1) active 2) inactive 3) healing II. Miliary infarct without stromal necrosis	I. Miliary infarct with stromal necrosis II. Massive infarction
Reversibility of process	Reversible	Irreversible	Irreversible
End result	Restitution to normal	Fibrous scar	Fibrous scar

tomical responses listed are not restricted to myocardial ischemia due to coronary atherosclerosis but may result also from other disturbances of metabolism. Such a concept would explain, for instance, the occurrence of any of these lesions, including infarct necrosis in the absence of significant coronary atherosclerosis. It would also explain

* Some of these disturbances of metabolism do not elicit morphologic alterations of the muscle primarily, but rather attract acute or chronic inflammatory cells resulting in some variant of myocarditis. This subject, however, is beyond the scope of this paper.

why occlusions of coronary arteries, if compensated for by a collateral circulation, need not produce a significant degree of metabolic imbalance.

The process of focal myocytolysis as it appears about the borders of an infarct was described by Smith,[4] in 1904, as follows:

"At the border of myocardial necrosis from infarction there occurs, either from the influences about to lead to solution of the dead mass, or as part of the reactive inflammatory change, sarcolysis of the ultimate fibrils of muscle through at least a short extent of the muscle fibre just outside the necrotic mass. This sarcolysis leaves, as remnants of the original fibre, structures which indicate the prior existence of a sarcolemma sheath. . . . In this solution of the muscle fibrils it is not essential that the muscle nuclei should likewise disappear, although by karyolysis they may be destroyed. . . . Such persistent nuclei are surrounded by a small amount of hyaline myoplasm and constitute the spindle-cell elements interpreted as myogenous connective tissue by some, the cells thus formed staining as does connective tissue. The fine fibrillae or membranous remnants of the sarcolemma . . . in a passive sense may contribute themselves as part of the fibrillar substance of the resultant scar. . . . The ultimate destiny of the persisting nuclei with their cell-like protoplasmic investment cannot be definitely declared. . . . The relative absence of these nuclei in the cicatrizing lesions, and the presence of small groups of pigment granules which probably mark the original situation of such nuclei, and the appearance of karyolytic changes in late stages of the process of organization in this or that example, and of atrophic forms, all speak for their eventual disappearance."

In hearts with coronary atherosclerosis the graded anatomical responses, listed in Table III, are mirrored in and overlap the clinical gradients of angina pectoris, coronary failure,[20] and myocardial infarction. The various lesser histologic manifestations—that is, the reversible degenerations, focal myocytolysis, and miliary infarcts—probably correspond to the lesser sequelae of clinical coronary heart disease. Thus, singly or in combination, they constitute the anatomical concomitants of angina pectoris and coronary failure.

Angina pectoris has long been thought to reflect cardiac ischemia or, in the broader sense here presented, metabolic imbalance in the heart. As the degree of this imbalance becomes great enough, irreversible damage to the heart muscle must follow. Various regressive cardiac responses then occur, including slowly evolving focal myocytolysis as well as more rapid miliary infarction, without or with stromal necrosis. Although such foci of irreversible muscle degeneration are not obligatory with every attack of angina pectoris, they probably develop with each episode of prolonged cardiac pain and coronary failure. Small fibrous scars in these hearts bear witness to such attacks.

Focal myocytolysis is thus compatible with all degrees of coronary heart disease, that is, with cardiac infarcts of clinical dimensions as

well as with angina pectoris and with coronary failure. On the other hand, this lesion conceivably develops without any clinical symptomatology, even angina pectoris.

This lesion may be epitomized as a focus of selective disappearance of parenchymal elements from an intact stroma, without exudative or proliferative responses. By this definition lesions identical in principle with focal myocytolysis of the heart should occur in other organs when they are subjected to disturbances of metabolism of proper degree. However, because of the inability of the heart muscle to regenerate and because of its syncytial nature, cytolysis in the heart is more readily detected. In parenchymatous organs whose constituent cells can regenerate, such as the liver, the analogous process may heal by reconstitution without leaving a trace. If the cytolysis is severe enough, reconstitution may be inadequate or incomplete, resulting in stromal collapse and fibrosis. Many a picture of diffuse fibrosis in various parenchymatous organs may have its genesis in a process akin to focal myocytolysis of the heart.

Conclusions

Focal myocytolysis of the heart is a miliary lesion which is characterized by a loss of muscular syncytium, preservation of the stroma, absence of inflammatory reaction, and eventual fibrosis.

Focal myocytolysis of the heart differs from a miliary infarct which presents coagulative necrosis of muscle, often involves the stroma, has an active inflammatory reaction, but is not different in its eventual fibrosis.

Focal myocytolysis of the heart is slower in evolution than infarction.

Focal myocytolysis of the heart is due to a lesser degree of metabolic imbalance than is miliary infarct.

Focal myocytolysis of the heart is more common than miliary infarct.

The data on incidence and degree of focal myocytolysis in the heart are based on a survey done by Dr. Priscilla Dienes Taft.

REFERENCES

1. Lisa, J. R., and McPeak, E. Acute miliary infarction of the heart. *Arch. Int. Med.*, 1940, 65, 919–932.
2. Brown, M. G.; Fienberg, R., and Holzman, D. Focal necrotizing myocarditis without interstitial infiltration. *Circulation*, 1951, 4, 909–912.
3. Akert, K. Die Insulin-Myokardose. *Schweiz. med. Wchnschr.*, 1950, 80, 1010–1015.

4. Smith, A. J. On the histological behavior of the cardiac muscle in two examples of organization of myocardial infarct. *Univ. Pennsylvania M. Bull.*, 1904–05, 17, 227–234.

5. Gouley, B. A.; McMillan, T. M., and Bellet, S. Idiopathic myocardial degeneration associated with pregnancy and especially the puerperium. *Am. J. M. Sc.*, 1937, 194, 185–199.

6. Roesler, H., and Soloff, L. A. Report of a case of left ventricular failure with unusual anatomical changes in the myocardium. *Ann. Int. Med.*, 1935–36, 9, 477–487.

7. Reisinger, J. A., and Blumenthal, B. Myocardial degeneration with hypertrophy and failure of unknown cause. *Am. Heart J.*, 1941, 22, 811–824.

8. Engelhardt, H. T., and Bruno, F. E. Fiedler's myocarditis. Report of a case. *New England J. Med.*, 1943, 228, 222–224.

9. Buchbinder, W. C., and Saphir, O. Heart failure in subacute bacterial endocarditis. *Arch. Int. Med.*, 1939, 64, 336–347.

10. Weiss, S.; Stead, E. A., Jr.; Warren, J. V., and Bailey, O. T. Scleroderma heart disease. With a consideration of certain other visceral manifestations of scleroderma. *Arch. Int. Med.*, 1943, 71, 749–776.

11. Dolgopol, V. B., and Cragan, M. D. Myocardial changes in poliomyelitis. *Arch. Path.*, 1948, 46, 202–211.

12. Durlacher, S. H., and Winternitz, M. C. Studies on the relation of the kidney to cardiovascular disease. V. Lesions of the myocardium. *Yale J. Biol. & Med.*, 1941–42, 14, 269–278.

13. Scriba, K. Über die Morphologie der Herzmuskulatur bei experimenteller Beriberi. *Verhandl. d. deutsch. path. Gesellsch.*, 1938, 31, 343–347.

14. Grundmann, E. Histologische Untersuchungen über die Wirkungen experimentellen Sauerstoffmangels auf das Katzenherz. *Beitr. z. path. Anat. u. z. allg. Path.*, 1950–51, 111, 36–76.

15. Franz, G. Eine seltene Form von toxischer Myokardschädigung. *Virchows Arch. f. path. Anat.*, 1936–37, 298, 743–752.

16. Menne, F. R.; Jones, O. N., and Jones, N. W. Changes in the myocardium of rabbits from augmenting the heart rate mechanically and from induced hyperthyroidism. *Arch. Path.*, 1934, 17, 333–355.

17. Geiringer, E. Intimal vascularisation and atherosclerosis. *J. Path. & Bact.*, 1951, 63, 201–211.

18. Schlesinger, M. J. An injection plus dissection study of coronary artery occlusions and anastomoses. *Am. Heart J.*, 1938, 15, 528–568.

19. Darrow, D. C., and Miller, H. C. The production of cardiac lesions by repeated injections of desoxycorticosterone acetate. *J. Clin. Investigation*, 1942, 21, 601–611.

20. Freedberg, A. S.; Blumgart, H. L.; Zoll, P. M., and Schlesinger, M. J. Coronary failure. The clinical syndrome of cardiac pain intermediate between angina pectoris and acute myocardial infarction. *J. A. M. A.*, 1948, 138, 107–114.

[*Illustrations follow*]

LEGENDS FOR FIGURES

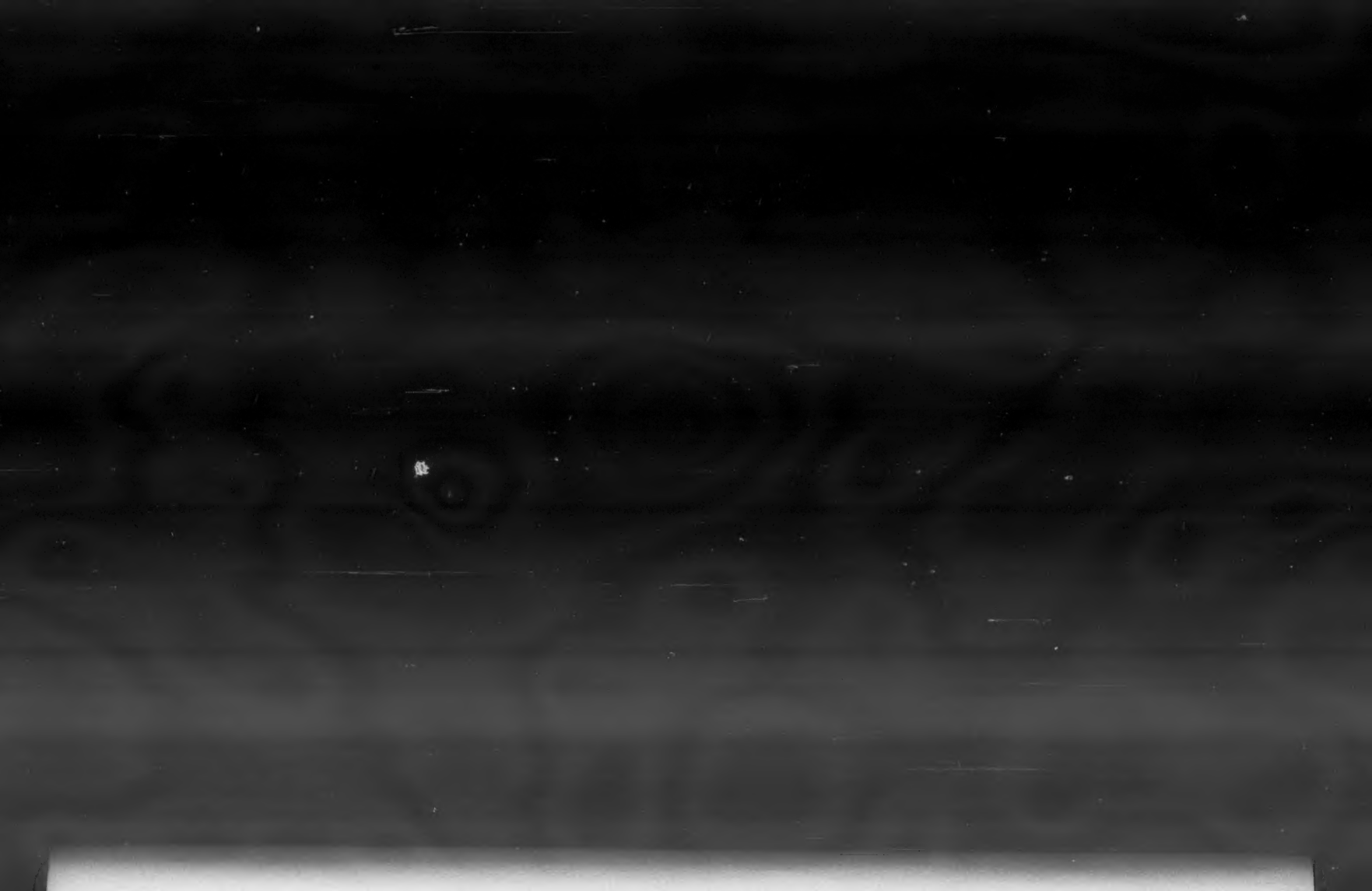
All sections were stained with hematoxylin and eosin.

FIG. 1. Earliest active stage of focal myocytolysis. Borders ill defined. Hydropic degeneration of muscle. Longitudinal section. × 185.

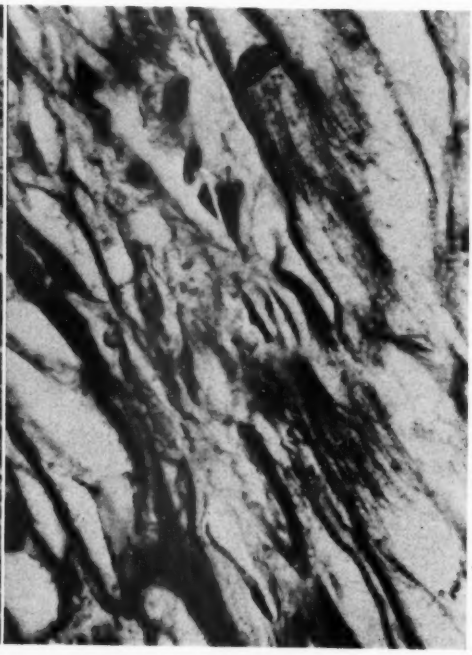
FIG. 2. High-power view of Figure 1. Active myocytolysis. Residual muscle nuclei intact. Muscle fibers frayed and blend imperceptibly with the rarefied cardiac stroma. Some nondescript remnants of the lysed muscle still contained within the sarcolemma. No stromal reaction or cellular exudate. × 475.

FIG. 3. Early active stage of focal myocytolysis. Three foci. Each focus abuts with at least one border upon a small interfascicular septum, accounting for the sharp borders over some part of their circumference. Borders are fuzzy where in contact with cardiac muscle. Cross section. × 185.

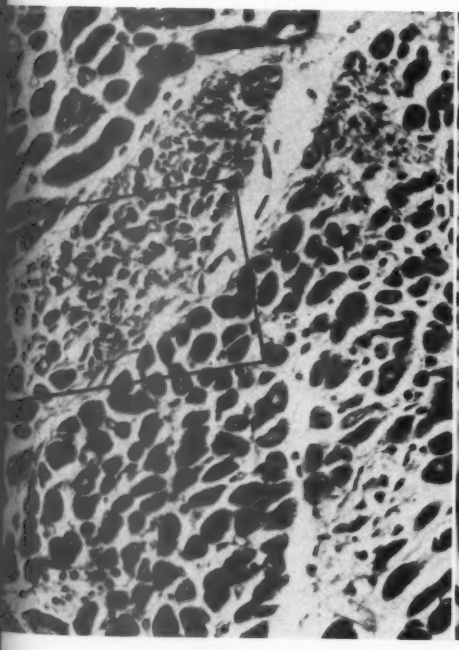
FIG. 4. High-power view of Figure 3. Disintegration of muscle fibers by fragmentation and myocytolysis. Seeming increase of nuclei is due to approximation of stromal and muscular elements rather than to proliferation or regeneration. Remaining muscle fibers small and of irregular shape, but nuclei are intact. No cellular exudation. × 420.



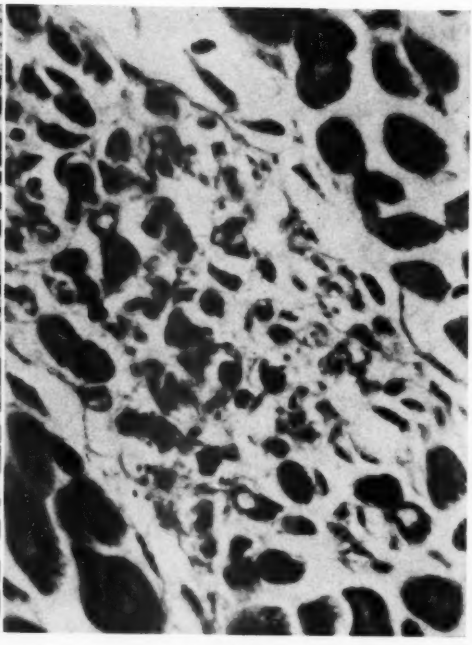
1



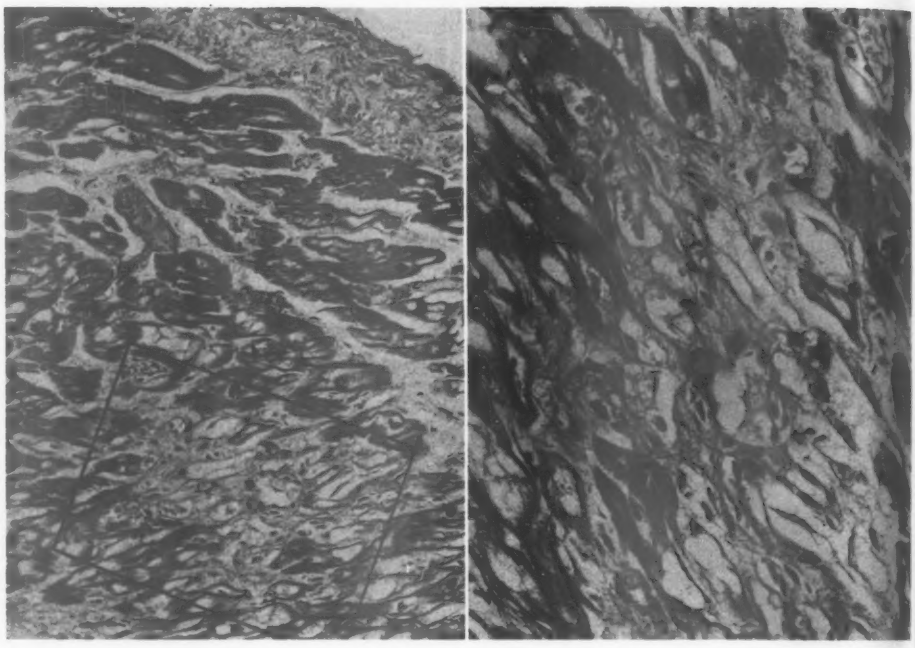
2



3



4



5 6

FIG. 5. Active stage of focal myocytolysis. Subendocardial focus in a papillary muscle. Centrifugal myocytolysis indicated by fuzzy borders. Longitudinal section. × 92.

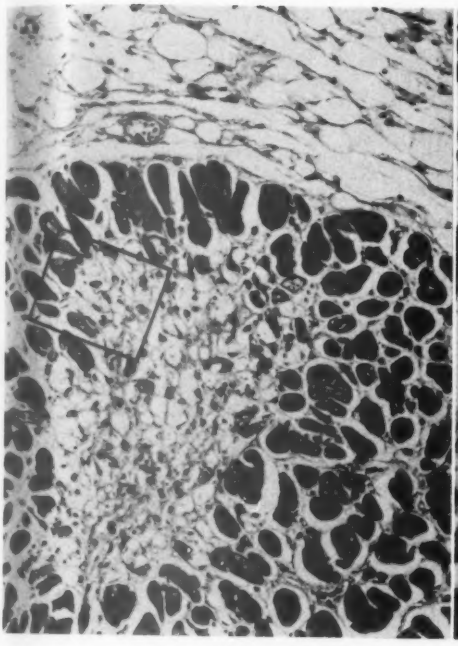
FIG. 6. High-power view of Figure 5. Active myocytolysis with fraying and splitting of involved muscle fibers. Rarefied sarcolemmal sheaths generally empty but a few contain either sparse myofibrils or a scalloped thin protein matter. No cellular exudation. × 185.

FIG. 7. Subsiding stage of focal myocytolysis. Subepicardial focus. Borders both sharply delineated and fuzzy. There is a certain resemblance between the empty stroma of focal myocytolysis and of the epicardial fat tissue. Cross section. × 92.

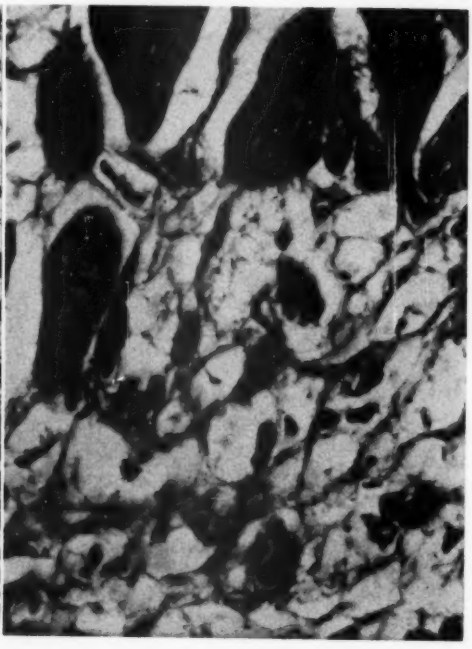
FIG. 8. High-power view of Figure 7. Detail from the sharp border. Isolated bits of unaltered muscle fibers inside sarcolemmal sheaths. Majority of sarcolemmal sheaths empty; a few contain foamy protein precipitate. Stroma intact. × 475.

FIG. 9. Subsiding stage of focal myocytolysis. Borders fairly quiescent. Empty cardiac stroma slightly collapsed. Cross section. × 92.

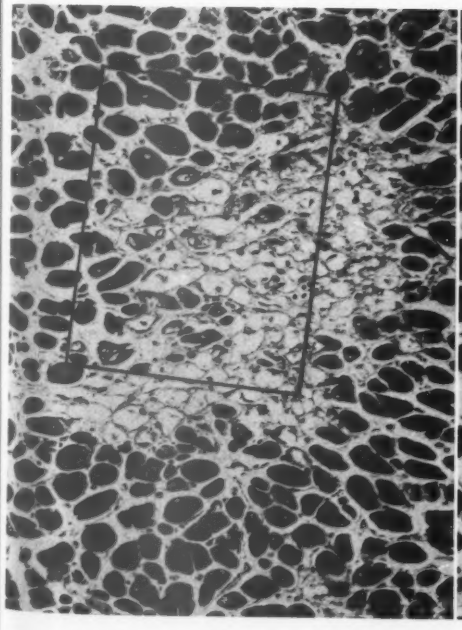
FIG. 10. High-power view of Figure 9. A few isolated fiber fragments exhibit disorderliness of myofibrils. No active myocytolysis. Stromal details well defined. Capillaries generally empty. × 185.



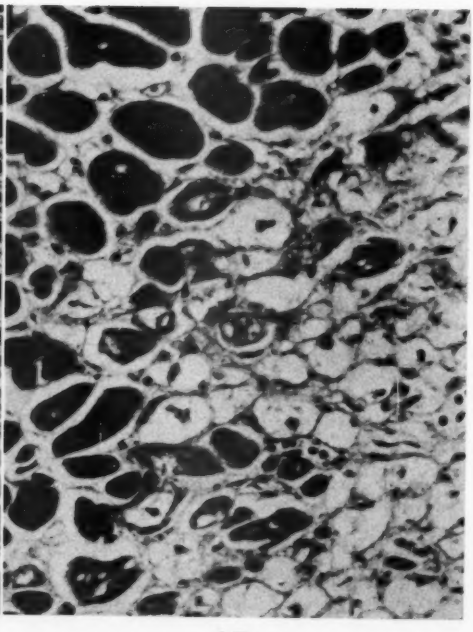
7



8



9



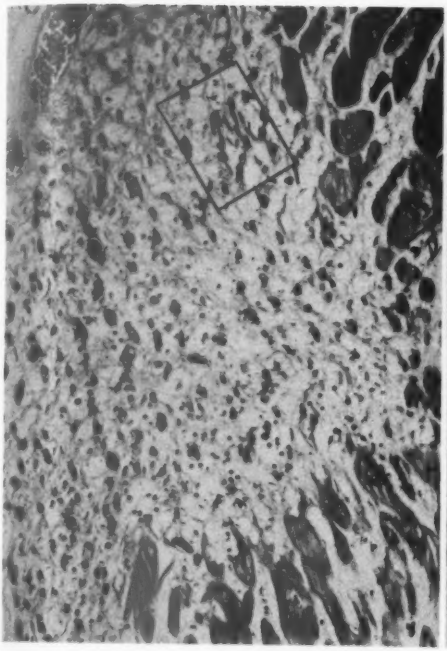
10

FIG. 11. Healing stage of focal myocytolysis. Edge of a larger focus (1500 by 700 μ). Some residual myocytolysis with a scattering of lymphocytes. Stromal reticulum empty and approximated. Capillaries engorged. × 94.

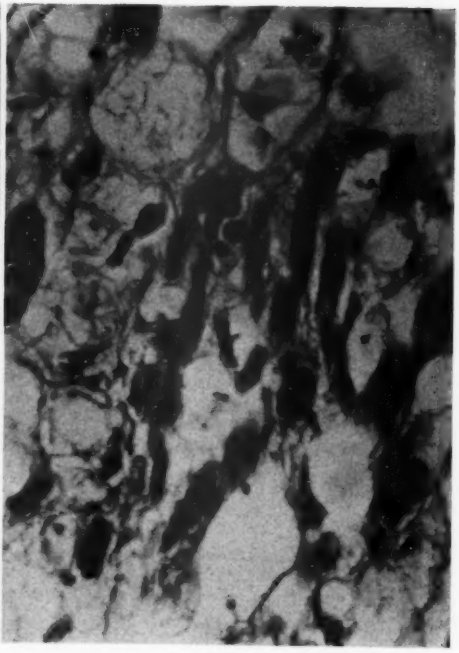
FIG. 12. High-power view of Figure 11. Attenuated remnants of cross-striated muscle. Other sarcolemmal sheaths either empty or containing a fine network of eosinophilic material. Congestion of capillaries. × 485.

FIG. 13. Healing and healed stage of focal myocytolysis. Two subepicardial foci. The upper focus is healing. Borders sharp. Periphery still composed of loose cardiac stroma. Central stroma collapsed. The lower focus is almost healed (fibrous scar). Borders quiescent. A few entrapped atrophic fibers toward 3 o'clock. Cross section. × 100.

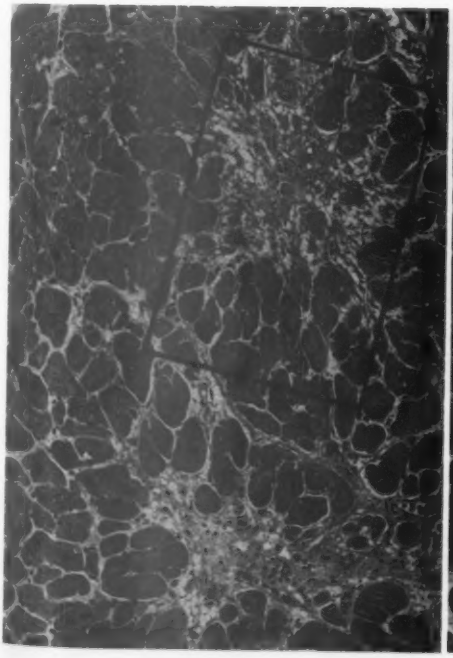
FIG. 14. High-power view of Figure 13. Healing focus. A few small but viable muscle fibers entrapped. Collapsed stroma in center. No myocytolysis. No stromal cell proliferation. No inflammatory cells. × 200.



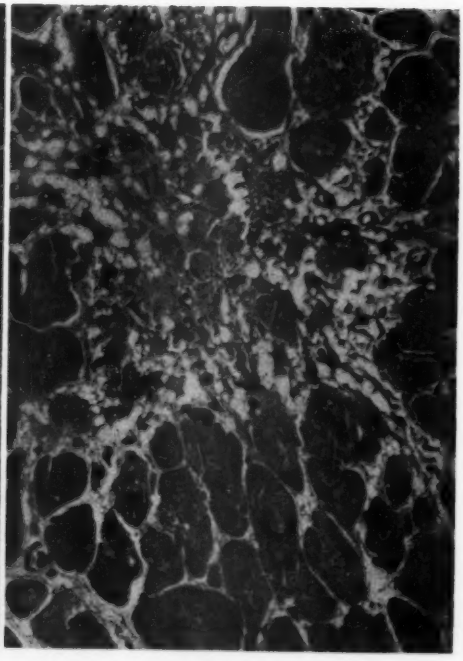
11



12



13



14

ATYPICAL CIRRHOSIS IN THE DUCK PRODUCED BY METHYLCHOLANTHRENE *

R. H. Riggon, M.D.

From the Laboratory of Experimental Pathology, University of Texas Medical Branch, Galveston, Texas

Methylcholanthrene has been used for the experimental production of tumors in the livers of mice.[1,2] It has been known for a long time that carcinogens will produce necrosis and cirrhosis in the livers of mammals.[3,4] Many investigators have produced experimental cirrhosis in the rat, the dog, and the rabbit with a variety of agents, among which may be mentioned high fat diets,[5] arsenates,[6] phosphorus,[7] copper,[8] chloroform,[9] manganese,[10] alcohol,[11] and carbon tetrachloride.[12] Apparently few histologic studies, however, have been made on the livers of birds following injury by chemical agents.

While we were studying the neoplasms[13-15] that occurred in white Pekin ducks following the subcutaneous, intramuscular, and dermal application of methylcholanthrene, a few birds developed a peculiar hepatic lesion which is reported at this time.

Methods and Materials

This study is based upon 18 adult white Pekin ducks as shown in Table I, 12 young ducks, 11 to 18 days of age, and 2 young adult birds weighing approximately 4 lbs. each. Methylcholanthrene in a 0.25 per cent acetone solution was dropped daily onto the skin of 8 ducks for 30 to 48 days. The daily amount varied from 0.5 to 2.0 ml. The area of the body beneath the right wing and the under-surface of this wing were treated. Methylcholanthrene used for intramuscular (7 ducks) and subcutaneous injections (3 ducks) was suspended in either sesame oil or mineral oil. Methylcholanthrene crystals were placed directly into the pectoral muscle through a surgical incision in one duck. The number of birds, their age, the method of treatment, and the time sacrificed are given in Table I and in the accounts of the different experiments.

The ducks were obtained from a commercial hatchery when 1 day old. They were fed Purina Duck Startena and Growena† and cracked corn. Food and water were available at all times. The younger birds

* This investigation was supported in part by a research grant P.H.S. C-1469 (C2) from the National Cancer Institute of the National Institutes of Health, Public Health Service.

Received for publication, August 30, 1954.

† Commercial foods made by the Ralston Purina Company, St. Louis, Mo.

were kept in batteries in the laboratory and the older ones in outside pens.

The majority of the ducks were sacrificed and necropsied immediately. A few birds were dead for less than 24 hours before they were examined. Sections of the liver were fixed in a 4 per cent solution of formaldehyde and paraffin sections were prepared. They were stained routinely with hematoxylin and eosin. Selected sections were stained by the following techniques: Heidenhain's aniline blue, Masson's trichrome (Foot's modification), Wilder's reticulum stain, Congo red, crystal violet, and Mayer's mucicarmine stain.

Experimental Findings

The livers were grossly normal from 10 ducks, 11 to 18 days of age, treated by the local application of methylcholanthrene in acetone to the skin daily for 30 days. They were sacrificed immediately after the last application of the carcinogen. Histologically, there were a few vacuoles within the hepatic cells. The livers from the 4 ducks similarly treated with acetone only showed a corresponding amount of fat. The absence of hepatic lesions attributable to methylcholanthrene in this first experiment may be accounted for by the small percentage of ducks that have shown hepatic damage following any form of treatment with methylcholanthrene. The absence of hepatic damage in these birds treated for only 30 days also may be related to the interval between the last application of the carcinogen and the time of death. All ducks showing significant hepatic damage either have died or were sacrificed after the 151st day of the experiment (Table I).

The hepatic lesion in the 18 ducks given methylcholanthrene, as shown in Table I, varied in degree. Sometimes the liver was grossly involved (Fig. 1), while other specimens showed only microscopic lesions.

The livers of 4 ducks, nos. 113, 117, 139, and 142, were characterized microscopically by a diffuse infiltration of fat throughout the organ. The remaining 14 ducks listed in Table I showed either a focal or diffuse hepatic fibrosis (Fig. 2). The primary process was a proliferation of reticulum following the degeneration of the hepatic cells. The space occupied by the hepatic cells was either partially or completely replaced by a homogeneous substance that stained pink with hematoxylin and eosin (Figs. 2, 3, and 4). This substance stained with Congo red in the same way that it did with hematoxylin and eosin, but with crystal violet it stained light purple. The hepatic cells showed some mucin when stained with Mayer's mucicarmine stain; however,

TABLE I

Ducks Treated with Methylcholanthrene and Showing Hepatic Lesions

Duck no.	Age	Injections				Length of experiment	Type of death	Remarks
		Route	No.	Amount	Age when injected			
21	*days* 16	S.C.	1	*ml.* 0.2*	*days* 16	*days* 273	D	Terminal septicemia from infected foot; liver enlarged, had lost weight
22	16	S.C.	1	0.2*	16	289	K	Female; focal yellow areas in liver
45	82	S.C.	3	1.0†	82,85,88	320	D	Female; liver enlarged
5	140	I.M.	1	1.0†	140	554	K	Female; focal yellow area in liver
32	115	I.M.	1	1.0†	115	554	K	Male; focal yellow area in liver
43	82	I.M.	3	1.0†	82,85,88	550	K	Female; liver pale yellow
47	82	I.M.	3	1.0†	82,85,88	266	D	Liver enlarged
113	14	I.M.	3	0.5†	14,19,25	581	K	Liver grossly normal
117	14	I.M.	3	0.5†	14,19,25	367	D	Liver grossly normal
330	50	I.M.	1	75 mg.‡	50	308	K	Chronic infection in wing joint; liver firm and yellow
139	33	Skin	67	Skin§	33	151	D	Liver normal grossly
142	33	Skin	67	Skin§	33,316,434	464	K	Applied 2nd time for 25 days when 316 days old; applied 3rd time for 30 days when 434 days old
143	13	Skin	48	Skin§	13,257,385	415	K	Applied 2nd time for 25 days when 257 days old; applied 3rd time for 30 days when 385 days old
145	13	Skin	48	Skin§	13	283	K	Liver enlarged, fibrotic; ascites, 250 ml.
262	11	Skin	30	Skin§	11	331	K	Female; ascites; liver firm
457	12	Skin	30	Skin§	12	294	K	Liver enlarged
480	12	Skin	30	Skin§	12	404	K	50% of liver fibrotic
488	12	Skin	30	Skin§	12	211	D	Died from hemorrhage from tumor

S.C. = subcutaneous; I.M. = intramuscular; D = died; K = sacrificed.

* 8 mg. of methylcholanthrene per ml. in sesame oil.

† 10 mg. of methylcholanthrene per ml. in mineral oil.

‡ Methylcholanthrene crystals.

§ 0.25% methylcholanthrene in acetone applied to the skin; 0.5 to 2.0 cc. used daily.

the stroma was negative for mucin. The stroma stained light green with the Masson trichrome stain and deep blue with the aniline blue stain (Figs. 7 and 8). A large amount of reticulum as shown by Wilder's stain occurred in these pink-staining areas (Figs. 5 and 6). These reticulum fibers usually were much wider and stained more deeply than the normal reticulum that is present in the same location in the hepatic lobule. There was also a marked increase in the number of these reticulum fibers. It is of interest to note that the quantity of reticulum, as demonstrated by this stain, did not fill the entire area that stained pink and blue by the techniques mentioned. This would suggest the presence of a large amount of some unidentified substance in these injured areas of the liver.

In some of the livers showing this degenerative process, a proliferation of endothelium-like cells was present in the hepatic sinuses around some of the small blood vessels and within the lumina of some of the vessels. Apparently, in the older lesions, this cellular reaction was absent. In those cases in which the degeneration was less extensive, small groups of hepatic cells remained (Fig. 4). The pink-staining, homogeneous material frequently surrounded these groups of hepatic cells. In such areas the hepatic sinuses were dilated; however, in those areas where the degeneration was more extensive, it frequently was difficult to demonstrate the sinusoids.

The characteristic hepatic lesion in ducks 22, 32, and 43 was a diffuse focal degenerative change similar to that previously described but primarily restricted to the area around the portal triads (Fig. 12). Few lymphocyte-like and plasma cells were present in such areas.

The liver of duck 143 was interesting since this was the only bird in the group that showed both an acute and chronic hepatic lesion. Methylcholanthrene was applied to the skin of this duck at three different times, the last being 30 days immediately before the bird was sacrificed. The chronic lesions in this duck were the same as those previously described and shown in Figures 2, 3, 4, and 5. The acute lesion was characterized by degeneration of the hepatic cells and an infiltration of neutrophils into the focal area (Fig. 10). Fat vacuoles were conspicuous in these degenerating hepatic cells. A moderate amount of straw-colored fluid was present in the abdominal cavity. Duck 142 was treated with methylcholanthrene in a manner similar to duck 143; however, the liver showed only a large amount of fat within the hepatic cells, with neither necrosis nor fibrosis (Fig. 11).

The maximum hepatic damage occurred in duck 145. This bird was observed to be weak and its legs were swollen on the 283rd day following the first application of methylcholanthrene to the skin. There were 250 ml. of straw-colored fluid in the abdominal cavity. The liver was approximately twice its normal size. The capsule was thick and the surface was slightly irregular (Fig. 9). On cut section it was yellowish brown and firm. The hepatic cells were absent in the greater portion of all sections studied. Such areas were represented by the pink-staining, acellular material previously described in ducks 143 and 480.

Control material for these observations consisted of more than 100 white Pekin ducks kept on the same commercial ration and examined for various purposes. The hepatic lesion described has not been found in any bird which did not receive methylcholanthrene.

Discussion

It would be preferable to have more material to illustrate the pathologic changes that develop in the liver preceding the occurrence of the pink-staining material that fills the spaces previously occupied by the hepatic cells. However, based upon the material we do have, it appears that the rate at which hepatic injury occurs is very slow when methylcholanthrene is either injected intramuscularly or applied locally to the skin of the duck in the manner and in the quantity used in this experiment. Methylcholanthrene may injure the hepatic cells either focally or diffusely. The former lesion may be located around the portal triads, while the latter may involve the hepatic cells throughout many of the lobules. The acute damage may be accompanied by a local infiltration of leukocytes. Following the degeneration of the hepatic cells, there occurs a proliferation of the reticulum. Accompanying this increase in reticulum there is also an accumulation of an unidentified material that stains pink with hematoxylin and eosin, light green with Masson's trichrome stain, and dark blue with aniline blue. Congo red stains this material light pink and crystal violet stains it light purple. Amyloid in mammals normally stains violet red by this latter technique. It would appear most likely from this study that this unidentified substance is collagen-like and not amyloid of the type found in mammals.

A hepatic lesion apparently identical with the one associated with the administration of methylcholanthrene was observed by Thomson and associates[16] in the livers of ducks, following the injection of a

vaccine containing killed tubercle bacilli in paraffin oil. The authors considered the lesion to be amyloid and obtained a positive reaction with Congo red.

In addition to this interesting degenerative change that occurred in the livers of ducks treated with methylcholanthrene, it is of considerable interest to note the apparent failure of bile ducts and hepatic cells to regenerate. This feature was not discussed in the paper by Thomson *et al.* in connection with the similar lesion which they produced in the duck.

In mammalian livers, as far as we know, there is always some regeneration following the injury produced by numerous agents. In the livers of these ducks there is only an increase in the reticulum that may be the forerunner of the unidentified collagen-like material which becomes so conspicuous in these specimens.

The absence of regeneration of hepatic cells and bile ducts in these livers and the absence of any neoplastic change following the administration of methylcholanthrene is interesting in view of the fact that hepatomas frequently are found in cirrhotic livers. It is generally agreed that cirrhosis precedes neoplastic formation when these two lesions are associated.[17,18] However, cirrhosis is not essential to the production of tumors.[19] The failure to observe neoplasms in the duck's liver may represent only a species variation, since tumors have been induced in the livers of mice treated with methylcholanthrene.[1]

Summary

The livers of white Pekin ducks treated intramuscularly, subcutaneously, and dermally with methylcholanthrene show either a focal or diffuse lesion characterized by a marked proliferation of reticulum without an accompanying hyperplasia of either hepatic cells or bile ducts such as one usually finds in mammalian livers injured by a variety of chemical agents. There may be ascites accompanying this atypical cirrhotic lesion in the duck.

The substance that replaces the degenerated hepatic cells is acellular and histologically is suggestive of amyloid; however, specific stains for amyloid were negative. A similar substance did not appear in other viscera.

REFERENCES

1. Strong, L. C. Genetic analysis of the induction of tumors by methylcholanthrene. Primary carcinoma of the liver following subcutaneous injection of methylcholanthrene in mice. *Arch. Path.*, 1944, 37, 131–135.
2. Andervont, H. B. The occurrence of spontaneous and induced pulmonary and liver tumors in strain C_3H mice. *Pub. Health Rep.*, 1939, 54, 1158–1168.

3. Davidson, J. The effect produced on the liver by application of coal tar to animals. *J. Path. & Bact.*, 1923, **26**, 127.

4. Twort, J. M., and Twort, C. C. Changes in the liver of mice following the ingestion of hydrocarbon oils. *Lancet*, 1932, **1**, 448–449.

5. Chaikoff, I. L., and Connor, C. L. Production of cirrhosis of the liver of the normal dog by high fat diets. *Proc. Soc. Exper. Biol. & Med.*, 1940, **43**, 638–641.

6. Von Glahn, W. C.; Flinn, F. B., and Keim, W. F., Jr. Effect of certain arsenates on the liver. *Arch. Path.*, 1938, **25**, 488–505.

7. Mallory, F. B. Phosphorus and alcoholic cirrhosis. *Am. J. Path.*, 1933, **9**, 557–567.

8. Mallory, F. B.; Parker, F., Jr., and Nye, R. N. Experimental pigment cirrhosis due to copper and its relation to hemochromatosis. *J. M. Research*, 1920–21, **42**, 461–490.

9. Schultz, E. W.; Hall, E. M., and Baker, H. V. Repair of the liver following the injection of chloroform into the portal system. *J. M. Research*, 1923–24, **44**, 207–230.

10. Findley, G. M. The experimental production of biliary cirrhosis by salts of manganese. *Brit. J. Exper. Path.*, 1924, **5**, 92–99.

11. Connor, C. L. Some effects of chronic alcohol poisoning in rabbits. *Arch. Path.*, 1940, **30**, 165–179.

12. Lamson, P. D., and Wing, R. Early cirrhosis of the liver produced in dogs by carbon tetrachloride. *J. Pharmacol. & Exper. Therap.*, 1926, **29**, 191–202.

13. Rigdon, R. H. Tumors produced by methylcholanthrene in the duck. Papilloma, squamous-cell carcinoma, and hemangioma. *A. M. A. Arch. Path.*, 1952, **54**, 368–377.

14. Rigdon, R. H. Fibroma arising from feather follicle of duck following local application of methylcholanthrene to skin. *Proc. Soc. Exper. Biol. & Med.*, 1953, **83**, 34–36.

15. Rigdon, R. H. Spontaneous regression of neoplasms: an experimental study in the duck. *South. M. J.*, 1954, **47**, 303–310.

16. Thomson, K. J.; Freund, J.; Sommer, H. E., and Walter, A. W. Immunization of ducks against malaria by means of killed parasites with or without adjuvants. *Am. J. Trop. Med.*, 1947, **27**, 79–105.

17. Winternitz, M. C. Primary carcinoma of the liver. *Johns Hopkins Hosp. Rep.*, 1916, **17**, 143–184.

18. Sugiura, K., and Rhoads, C. P. Experimental liver cancer in rats and its inhibition by rice-bran extract, yeast, and yeast extract. *Cancer Research*, 1941, **1**, 3–16.

19. Opie, E. L. The pathogenesis of tumors of the liver produced by butter yellow. *J. Exper. Med.*, 1944, **80**, 231–246.

[*Illustrations follow*]

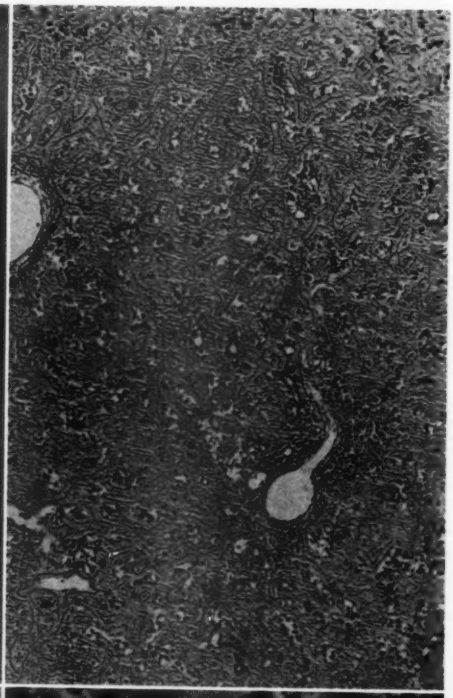
LEGENDS FOR FIGURES

FIG. 1. Duck 480. The skin of the body beneath the right wing was treated with a 0.25 per cent acetone solution of methylcholanthrene daily for 30 days, beginning when the bird was 12 days of age. The duck was sacrificed 404 days later. Grossly, the liver showed focal areas of fibrosis, involving approximately 50 per cent of the organ.

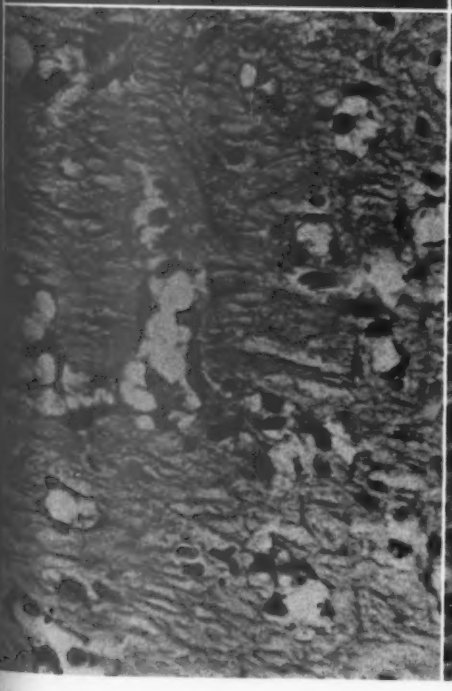
FIG. 2. Duck 480. The hepatic cells have been replaced by pink-staining stroma. Of note is the absence of any hyperplastic nodules. Hematoxylin and eosin stain. × 100.

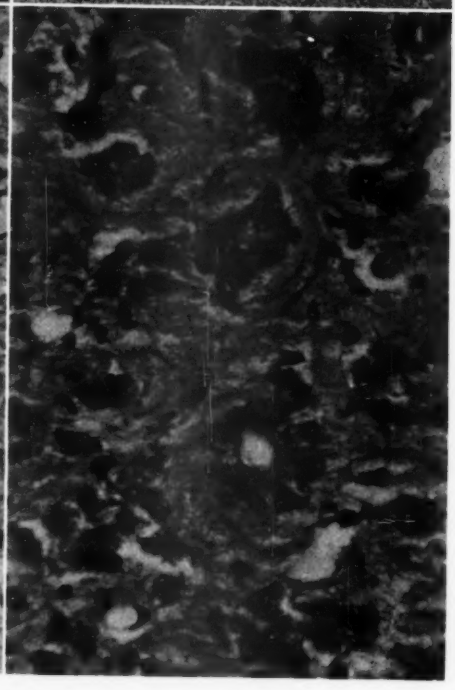
FIG. 3. Duck 480. There are a few reticulo-endothelial cells lining the sinuses. Of note is the absence of hepatic cells. Hematoxylin and eosin stain. × 473.

FIG. 4. Duck 145. Groups of hepatic cells are still present in this area of liver. The degree of injury is less severe than that shown in Figure 3. There is a large amount of pink-staining stroma surrounding these groups of hepatic cells. The skin of this duck was treated daily for 48 days. The duck was sacrificed 283 days later. Hematoxylin and eosin stain. × 473.



2





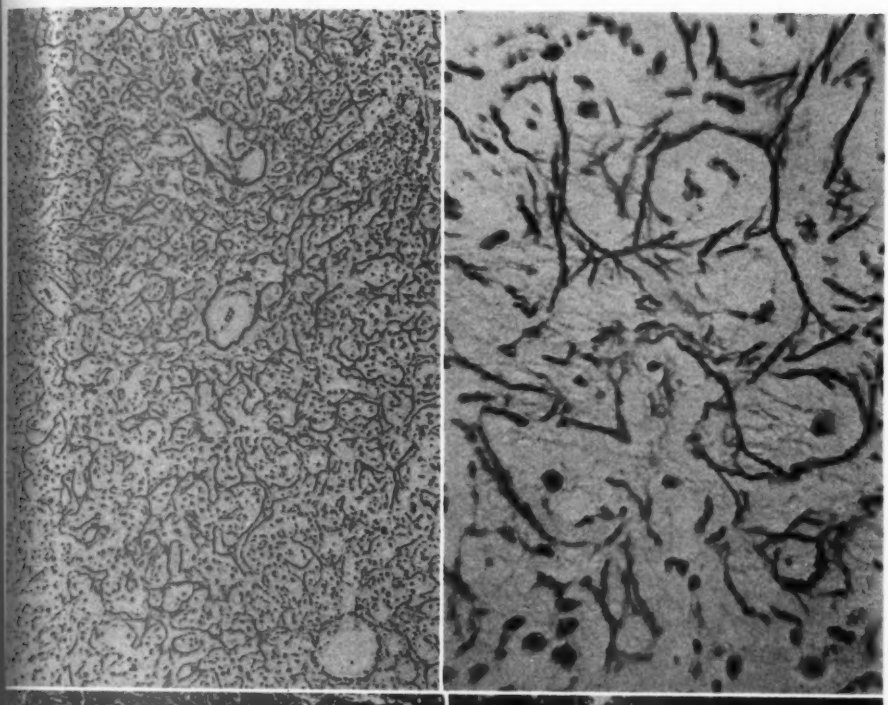
4

FIG. 5. Duck 480. There is a diffuse increase in reticulum throughout the degenerated area of liver. This is essentially the same area of tissue as shown in Figures 2, 3, 5, and 6. Wilder's reticulum stain. × 100.

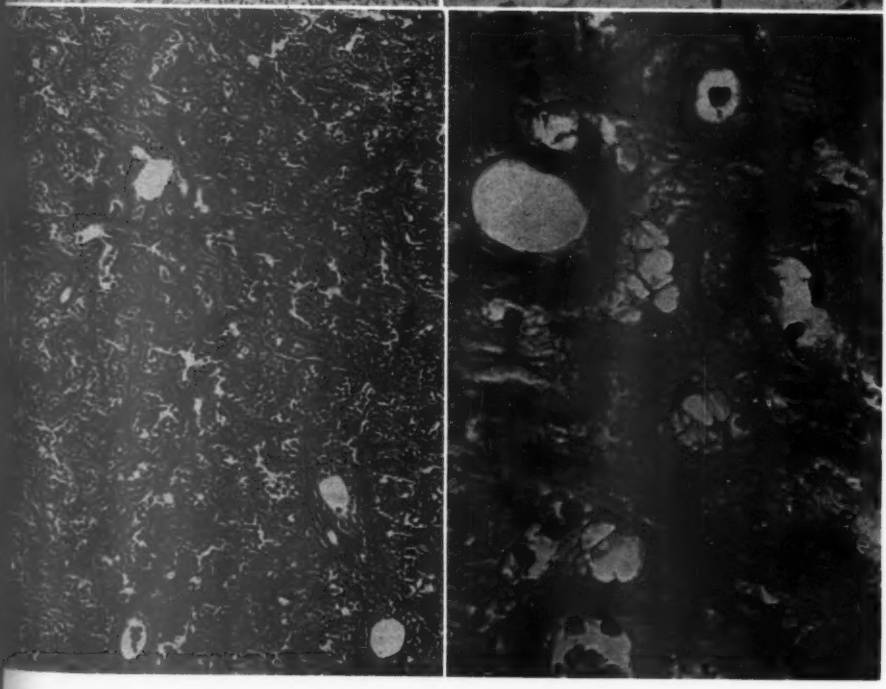
FIG. 6. Duck 480. The fibers of reticulum are much wider than normal. Only a portion of the pink-staining tissue replacing the hepatic cells gives a positive stain for reticulum. Wilder's reticulum stain. × 473.

FIG. 7. Duck 480. Essentially the same area of degeneration as shown in Figure 5. The area stains deeply with aniline blue. Heidenhain's aniline blue stain. × 100.

FIG. 8. Duck 480. Same as Figure 7. × 473.



6



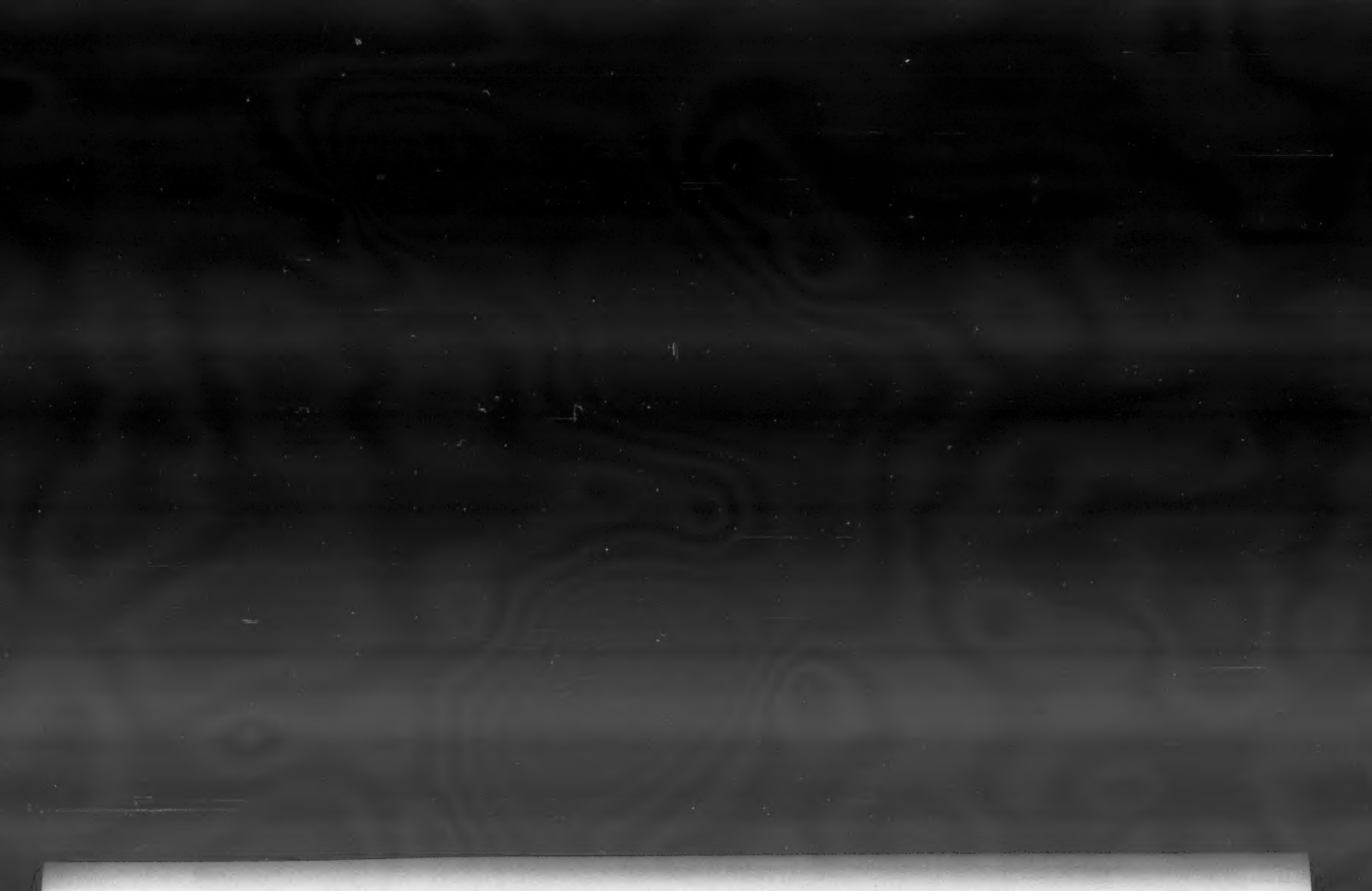
8

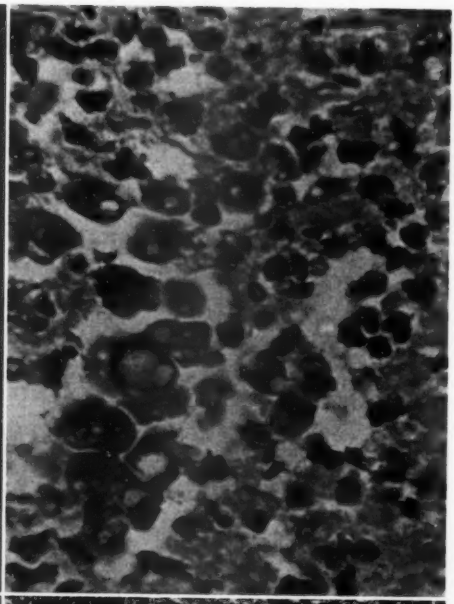
FIG. 9. Duck 145. The greater part of the right lobe and part of the left lobe of the liver are fibrotic. The capsule is markedly thickened. Figure 4 may be seen for histologic lesions.

FIG. 10. Duck 143. The skin of this duck was treated on three different occasions, the last time for 30 days immediately before the bird was sacrificed. Focal areas of acute degeneration are present in the liver as well as typical chronic lesions. Hematoxylin and eosin stain. × 688.

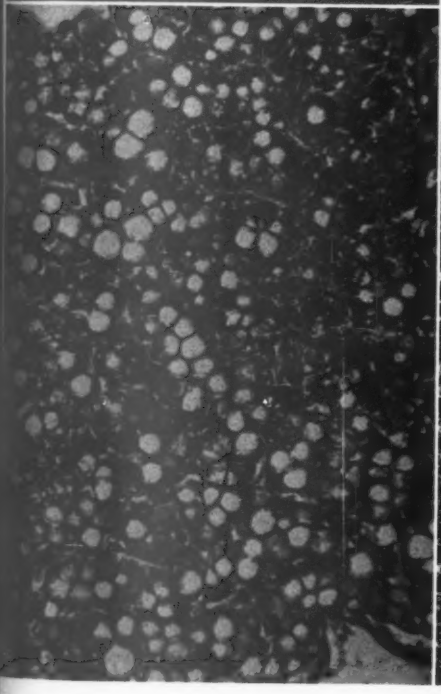
FIG. 11. Duck 142. This duck was treated similarly to duck 143, Figure 10. The liver shows a very extensive infiltration with fat. Apparently such a lesion may precede fibrosis. Hematoxylin and eosin stain. × 200.

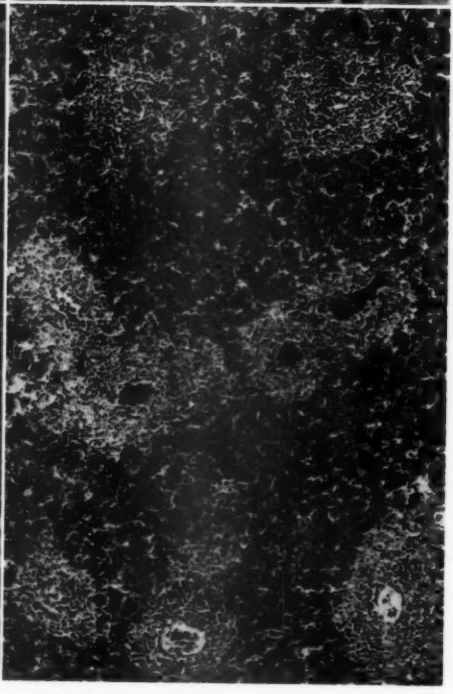
FIG. 12. Duck 45. Three subcutaneous injections of 1.0 ml. of methylcholanthrene in mineral oil each were given on the 82nd, 85th, and 88th experimental days. Death occurred on the 320th day. The focal areas of degeneration usually occur around the portal triads. Few lymphocytes and plasma cells may infiltrate the area. Hematoxylin and eosin stain. × 100.





10





12

THE PATHOLOGY OF INFECTIOUS SEROSITIS OF DUCKS*

E. Dougherty, 3rd, V.M.D.; L. Z. Saunders, D.V.M., and E. H. Parsons, Jr., B.S.
From the New York State Veterinary College Duck Disease Research Laboratory, Eastport, N.Y. (Dr. Dougherty and Mr. Parsons), and the Brookhaven National Laboratory, Upton, N.Y. (Dr. Saunders)

During the past 4 years, 7,155 ducks have been necropsied at the Duck Disease Research Laboratory. Of these, 2,216 were affected with a pathologic entity which we have designated infectious serositis. This disease represented 55 per cent of the accessions of birds 2 to 8 weeks of age. It is economically the most serious disease affecting domestic ducks in the United States. Several duck ranchers on Long Island were questioned in an attempt to establish when the disease was first observed. Some could recall its occurrence on their farms over a decade ago, but these recollections were based only on clinical symptoms.

Two diseases are recorded in the literature which are clinically indistinguishable from infectious serositis. The first is "anatipestifer infection" (new duck disease), described by Hendrickson and Hilbert[1] from Long Island in 1932. The second is the "duck septicemia" described by Graham, Brandly, and Dunlap[2] from Illinois in 1938. Both of these may be the same disease described here; however, since neither report contains a detailed description of the lesions, anatomical comparisons cannot be made.

Clinical Features

During the growing season, February to November, most Long Island duck farms have a series of flocks ranging from 1 through 8 weeks of age on the premises. Infectious serositis usually breaks out first in one of the older groups (6 to 8 weeks of age). The disease eventually descends through the various flocks until birds about 10 days of age are affected. The assembly line system of moving the ducks every 4 to 7 days into the quarters vacated by the next older group accelerates the spread of the infection.

A mild cough accompanying white or greenish white diarrheal discharge are the first symptoms. The feathers around the vent are frequently stained green. Older birds develop a slow tremor of the head, locomotor incoordination, and finally lie on their backs and paddle

* Part of this study was carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.
Received for publication, October 2, 1954.

convulsively with their legs. Many of the birds so affected die of inanition. The course of the disease in a flock may vary from 1 to 4 weeks. Individual birds die in 1 to 7 days after symptoms appear. In flocks 2 weeks of age birds die after only 1 or 2 days of illness. In older flocks the birds may be ill 6 to 7 days before dying.

Morbidity as indicated by anorexia and diarrhea is very high in flocks affected with serositis, often approaching 100 per cent. Mortality is highest in younger flocks (under 4 weeks of age). Mortality rates in these age groups vary from 20 to 65 per cent. In older ducks mortality is usually under 20 per cent.

Adverse environmental conditions often predispose to outbreaks of the disease. Severe climatic changes or sea tides backing up into the ducks' fresh water streams have been noted to precede some of the outbreaks.

Pathologic Findings

The distribution of gross lesions in 100 consecutive necropsies on birds affected with serositis is recorded in Table I. The following is a description of the pathologic changes in each organ.

Table I

Distribution of Gross Lesions in 100 Ducklings (74 Females and 26 Males)

Splenomegaly	89
Air sac inflammation	87
Pericarditis	85
Perihepatitis	75
Salpingitis	24*

* Represents 32.4% of females.

Heart. Fibrinous pericarditis was one of the more common and striking lesions (Fig. 1). The pericardium was often adherent to the heart and in some cases also to the thoracic wall. Histologically, the inflammation in most cases did not extend beyond the epicardium (Fig. 5). In some cases the superficial portion of the myocardium was involved also, and inflammatory cells infiltrated the spaces between the myocardial fibrils (Fig. 6). The cellular infiltrate consisted predominantly of large mononuclear cells, with a small percentage of heterophilic leukocytes.

Liver. The liver was enlarged and covered by a yellowish white membrane (Fig. 4). Histologically, this membrane contained fibrin and numerous inflammatory cells, similar to those in the pericardium (Fig. 7). In addition there were numerous fibroblasts, which tended to organize the exudate early in the course of the disease. At necropsy the exudate was often sufficiently organized to slip off as a membranous cover when the liver was handled. The liver was sometimes affected by periportal infiltration of inflammatory cells. The blood vessels were congested.

Spleen. The spleen was enlarged to three to five times the normal volume. Its color was pale and the surface mottled, giving it a lobular appearance somewhat resembling a pig's liver (Fig. 3). Fibrin was often present on the serous surface.

Kidneys. The blood vessels in the kidneys were unduly prominent (Fig. 2). Fibrinous exudate was present on the ventral surface. Histologically, passive congestion was the only change seen. All of the veins were distended, the congestion being particularly prominent in the smaller vessels.

Oviducts. One or both oviducts were distended throughout their length by caseous exudate in about one third of the females (Fig. 9). Histologically, the submucosa was infiltrated by inflammatory cells which extended into the mucosal folds (Fig. 10). The lumen was distended by exudate which was predominantly cellular and contained little fibrin. The cells were almost all large mononuclear phagocytes. The salpingitis was considered a part of the pathologic picture, because pericardial or hepatic lesions were present in all birds with salpingitis. Salpingitis without accompanying lesions in other organs has not been found in this laboratory in young ducks.

Alimentary Canal. The blood vessels in the serosa of the intestine were unduly prominent. Mucopurulent exudate sometimes was present in the lumen of the intestine.

Nasal Sinuses. Mucopurulent exudate often was present in the nasal sinuses.

Air Sacs. Grossly, the air sac membranes were thickened and opaque. Histologically, there was distention by fibrinous and cellular exudate to approximately twice the normal thickness (Fig. 8). The inflammatory cells were predominantly large mononuclear phagocytes. In birds in which the disease had apparently run a less acute course, some of the mononuclear cells had coalesced to form giant cells. Fibroblastic proliferation also was present in the more chronic cases. The gross appearance was similar to that described in chronic respiratory disease of chickens; however, the lymphofollicular lesions of chronic respiratory disease were not seen.[3]

Lungs. No significant changes were found in the lung sections, nor were there any in the trachea and bronchi. The inflammation of the air sacs did not extend to the pulmonary parenchyma.

Central Nervous System. Fibrinous cerebrospinal meningitis was present in all birds with nervous symptoms, and in some which died rapidly without well defined symptoms (Figs. 11 and 12). In a few specimens the exudate in the leptomeninges was thick enough to be

recognizable grossly as soon as the dura mater was incised. The meningitis extended from the forebrain to the end of the spinal cord. The inflammatory cells were studied in smears of cerebrospinal fluid taken at necropsy from freshly killed birds, as well as in tissue sections. Large mononuclear phagocytes predominated, but heterophils were always present in numbers estimated at 5 to 25 per cent of the inflammatory cells. Inflammatory changes in the brain were slight, and were seen only in the peripheral tissue adjacent to the meninges, where a few vessels with perivascular cuffing were found (Fig. 13). Pyknosis of some of the motor neurons was seen in several spinal cords (Fig. 14). No neuronophagia or myelin sheath degeneration was seen in the central nervous system. Inflammatory changes were present in a number of lumbar spinal nerve roots, but the inflammation did not extend to the sciatic nerves.

The extensive meningitis in our birds distinguishes serositis from the virus encephalitis of wild ducks described by Rosenow.[4] In addition, none of the visceral lesions described here were present in the wild ducks.

Peripheral Blood. Wright-stained blood smears were examined from 120 birds and spleen impression smears from over 1,000 birds. No duck plasmodium, leukocytozoon, or other blood-cell parasite was ever encountered.

Interpretation of Lesions

The pathologic picture was one of a generalized fibrinous inflammation of the serous membranes. This attribute was shared alike by the meningeal, pleural, pericardial, and peritoneal surfaces. The inflammation showed little or no tendency to extend from the membranes to the underlying parenchymatous organs. It is likely that the nervous symptoms were referable to the lesions in the central nervous system. The congestion observed in the liver, spleen, intestine, and kidneys was probably due to cardiac insufficiency.

Although there were a few differences, the pathologic picture as a whole was similar in many respects to that of sporadic bovine encephalitis.[5]

Differential Diagnosis

Infectious serositis can be distinguished from other diseases of ducks by the following features:

Virus Hepatitis of Ducks. Petechial and ecchymotic hemorrhages on the surface of the liver are characteristic of virus hepatitis. These were not seen in infectious serositis. On the other hand, the lesions of infectious serositis were not seen in uncomplicated hepatitis.

Fowl Cholera. Differentiation between the peritoneal form of cholera and infectious serositis must be made by cultural identification of the *Pasteurella multocida* organism; however, the serosal exudate of fowl cholera is yellow, while that of serositis is yellowish white. The petechial hemorrhages on the epicardium and the focal necrosis of the liver which characterize acute fowl cholera were not seen in infectious serositis.

Duck Plague. In duck plague, as in serositis, there is pericarditis, peritonitis, and salpingitis. However, the widespread hemorrhages described in plague have not been seen in serositis, and there is no mention of neurologic lesions in the description of plague.[6]

Discussion

Because of the nature of the lesions, which affected chiefly the serous membranes, the name serositis was chosen for this disease. Hjäre and Wramby[7] have previously applied this term to a disease of swine with similar widespread exudative changes. In the absence of etiologic information we are naming the disease on the basis of its morbid anatomical features.

We have been able to transmit the disease to ducks by the administration of suspensions of ground spleen, liver, and serosal exudates, thus establishing its infectious nature. Intratracheal and intraperitoneal inoculations have been successful in 95 of 120 attempts. The etiologic agent has not yet been determined. The non-suppurative character of the inflammation is suggestive of a non-bacterial etiology. The similarity of the pathologic picture to that of sporadic bovine encephalitis suggests that a psittacoid organism may be involved.

Summary

Infectious serositis of ducks is an epizootic disease with a high morbidity and a mortality of 5 to 60 per cent. It is characterized anatomically by cerebrospinal meningitis, pericarditis, and perihepatitis. The infection can be transmitted by inoculation of diseased tissue, but the nature of the etiologic agent is not known.

We are indebted to Robert F. Smith for the photographic illustrations.

REFERENCES

1. Hendrickson, J. M., and Hilbert, K. F. A new and serious systemic disease of young ducks with a description of the causative organism, *Pfeifferella anatipestifer*. *Cornell Vet.*, 1932, **22**, 239–252.
2. Graham, R.; Brandly, C. A., and Dunlap, G. L. Studies on duck septicemia. *Cornell Vet.*, 1938, **28**, 1–8.

3. Johnson, E. P. The specificity of lymphofollicular lesions in the diagnosis of chronic respiratory disease. *Cornell Vet.*, 1954, **44**, 230–239.
4. Rosenow, E. C. Studies on the relation of a neurotropic streptococcus and virus to epizootic encephalitis of wild ducks. *Cornell Vet.*, 1943, **33**, 277–304.
5. Menges, R. W.; Harshfield, G. S., and Wenner, H. A. Sporadic bovine encephalomyelitis. I. The natural history of the disease in cattle. *Am. J. Hyg.*, 1953, **57**, 1–14.
6. Bos, A. Weer nieuwe gevallen van eendenpest. [Again new cases of duck plague.] *Tijdschr. v. diergeneesk.*, 1942, **69**, 372–381.
7. Hjäre, A., and Wramby, G. Om fibrinös serosa-ledinflammation (Glässer) hos svin. [Fibrinous serosal and joint inflammation in swine.] *Skandinav. vet. tidsskr.*, 1942, **32**, 257–289.

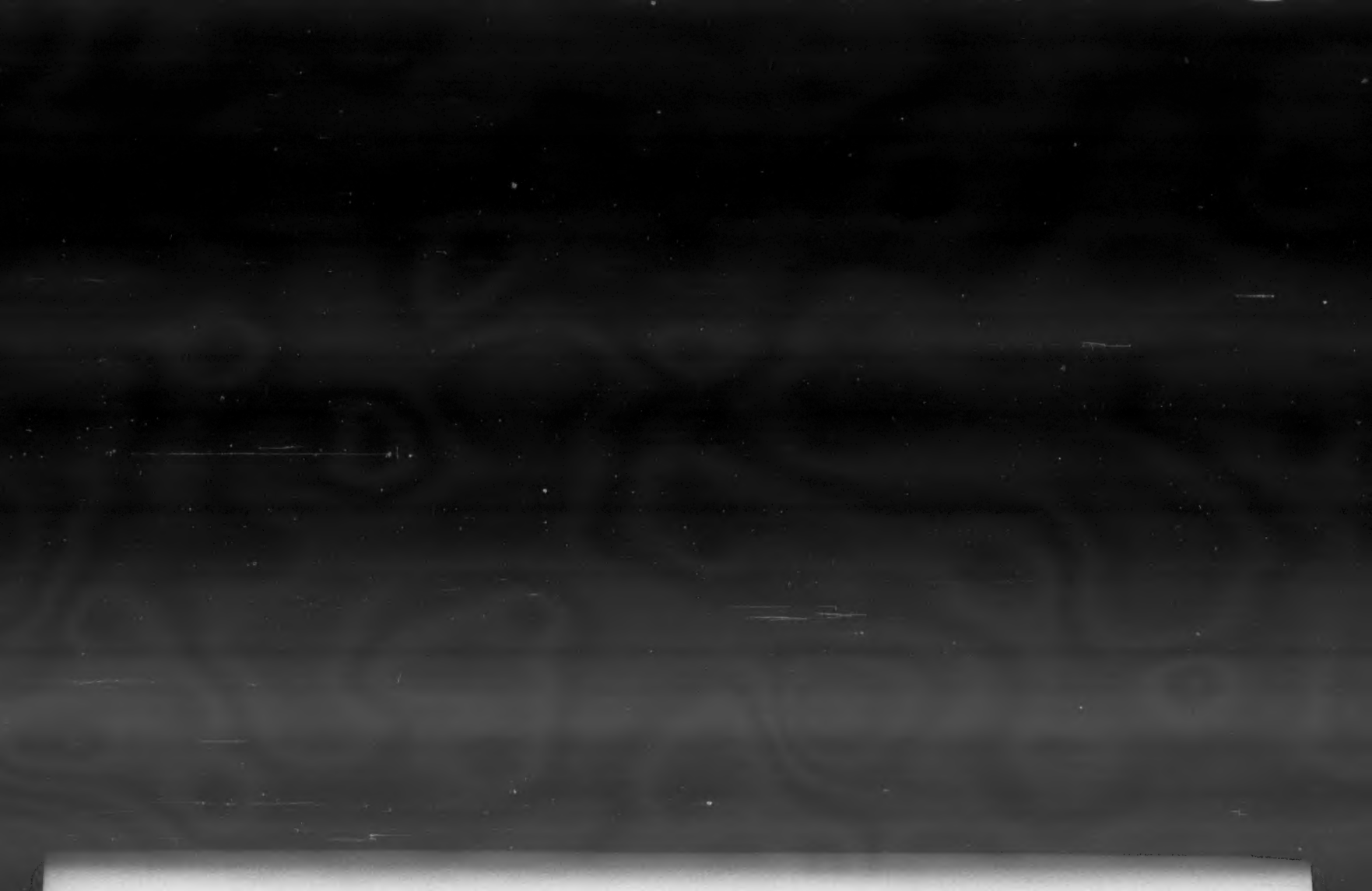
LEGENDS FOR FIGURES

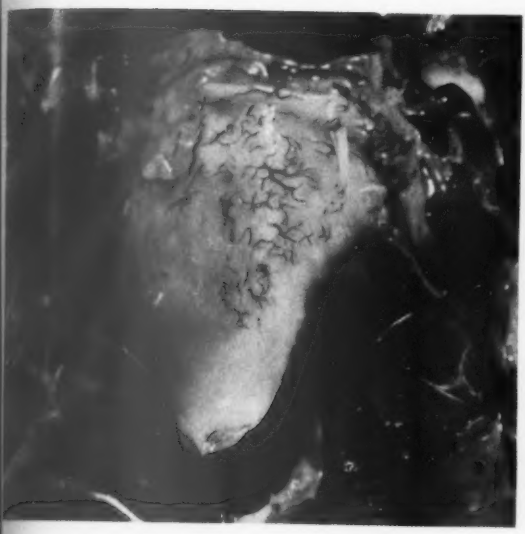
FIG. 1. Fibrinous pericarditis in a 3-weeks-old duckling.

FIG. 2. Ventral surface of the kidneys, with passive congestion.

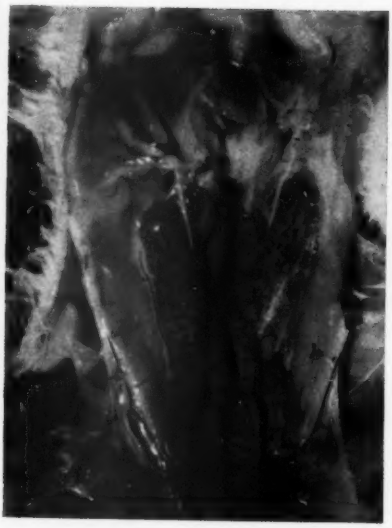
FIG. 3. Dorsal view of the spleen with mottling visible through the fibrin on the serous surface.

FIG. 4. Fibrinous perihepatitis and pericarditis.

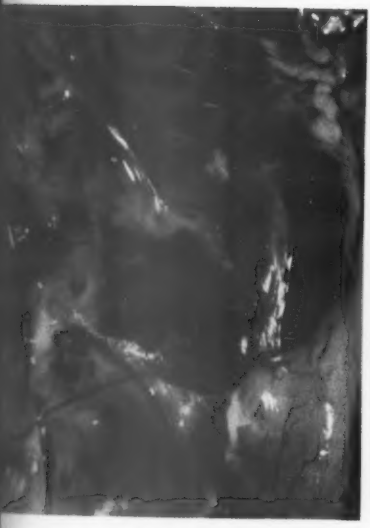




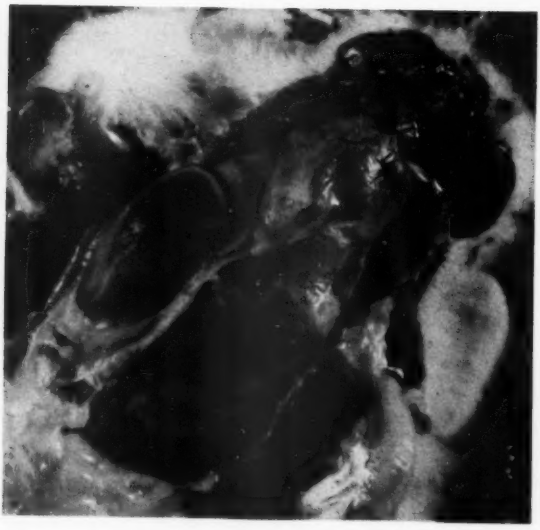
1



2



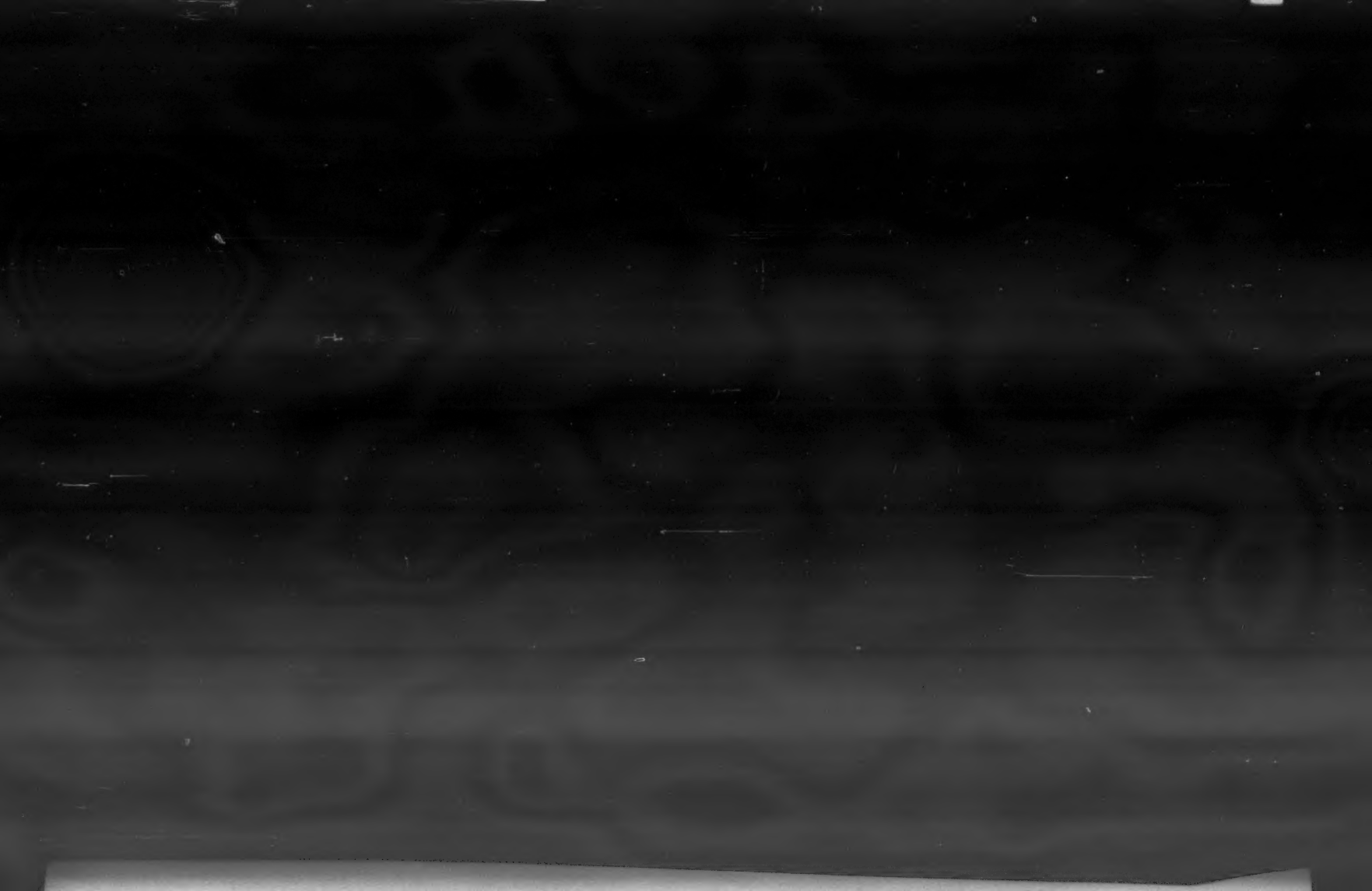
3

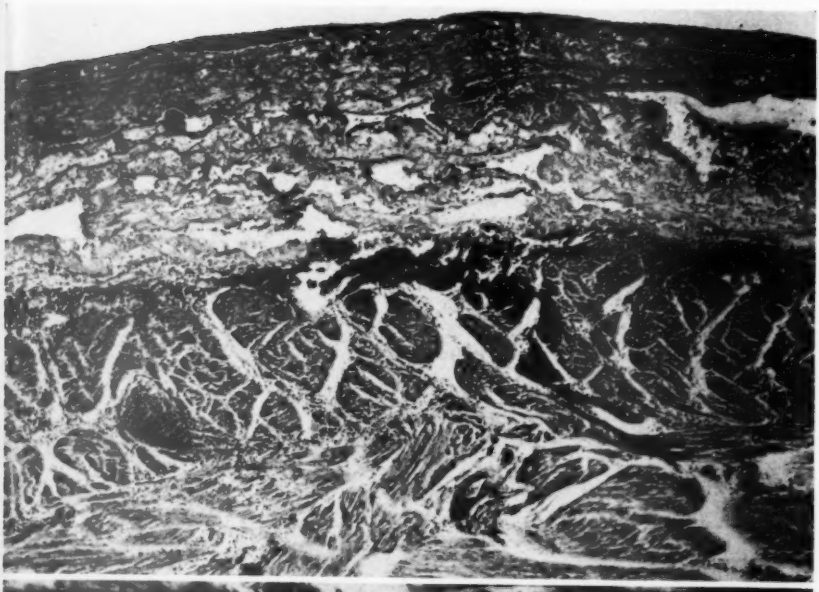


4

FIG. 5. Pericarditis. A thick layer of exudate on the epicardial surface. × 50. This and all subsequent sections were stained with hematoxylin and eosin.

FIG. 6. Myocarditis. Inflammatory cells and edema between myocardial fibers. × 220.





5

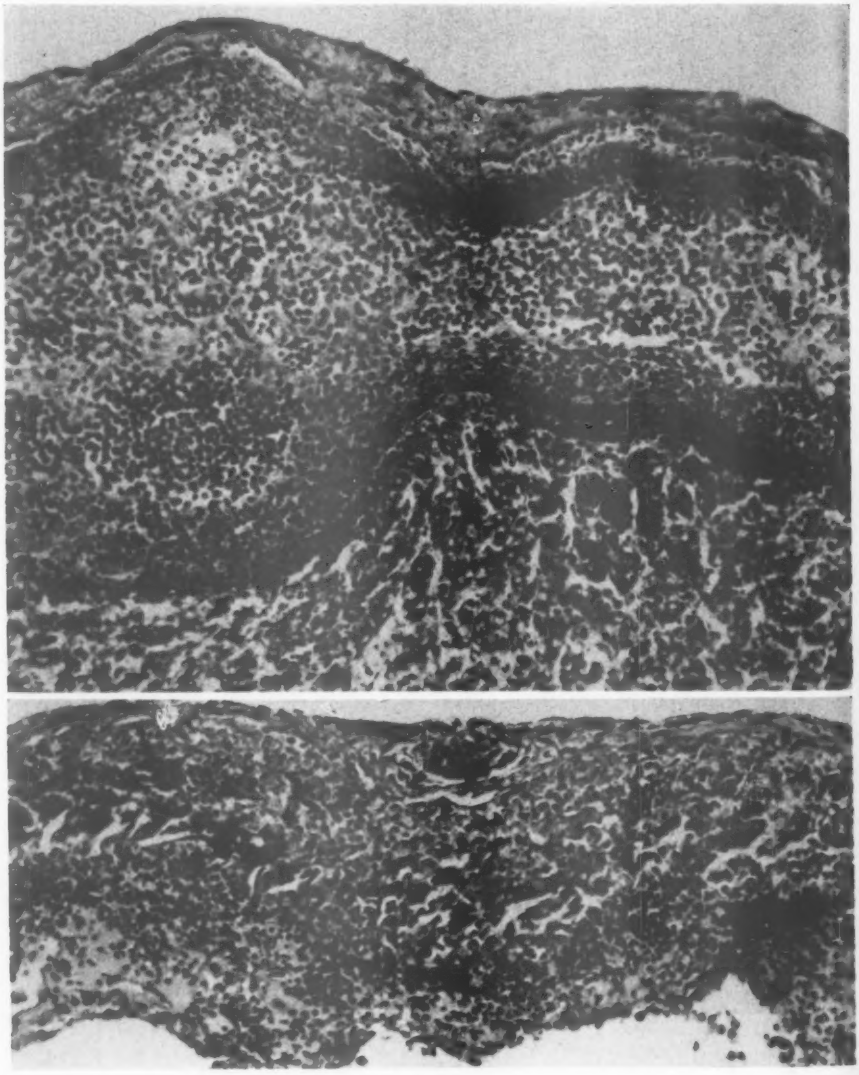
6

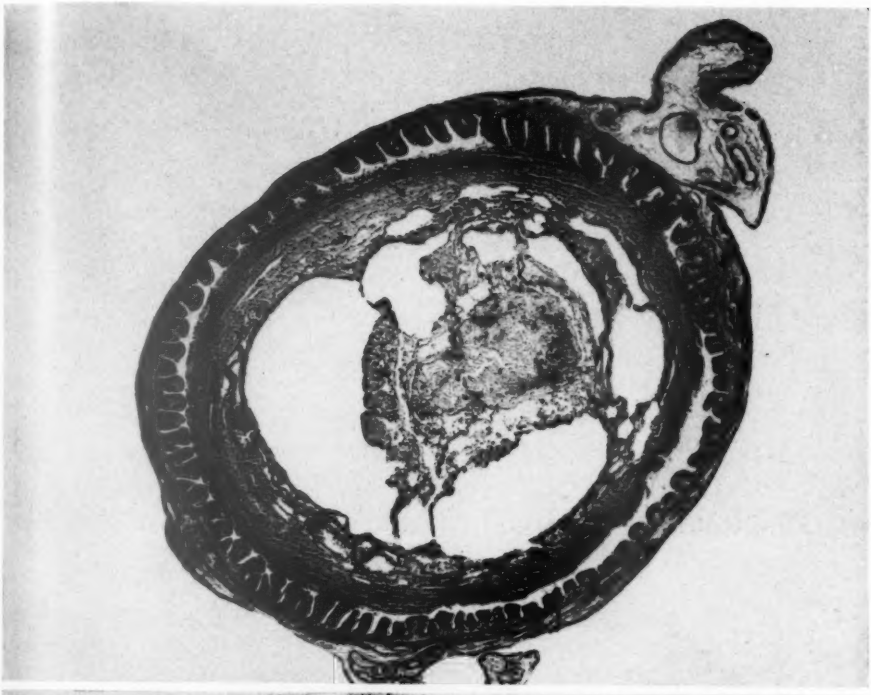
FIG. 7. Liver with thick layer of exudate on its peritoneal surface. × 220.

FIG. 8. Air sac thickened by infiltration with fibrinous and cellular exudate. × 220.

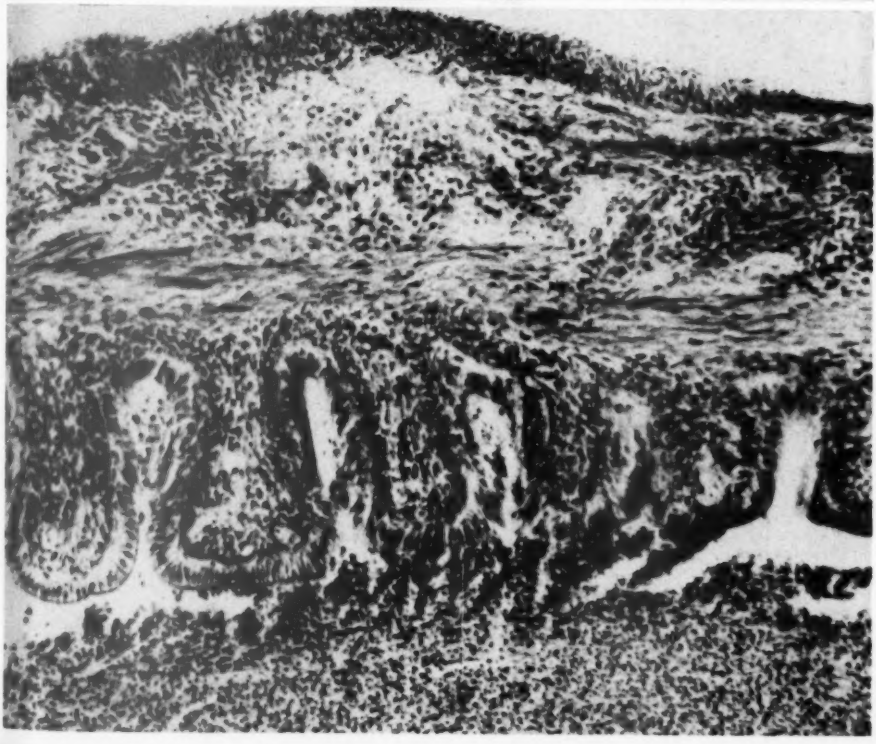
FIG. 9. Oviduct containing caseous exudate. × 18.

FIG. 10. Salpingitis. Infiltration of the submucosa and mucosal folds with inflammatory cells. × 185.



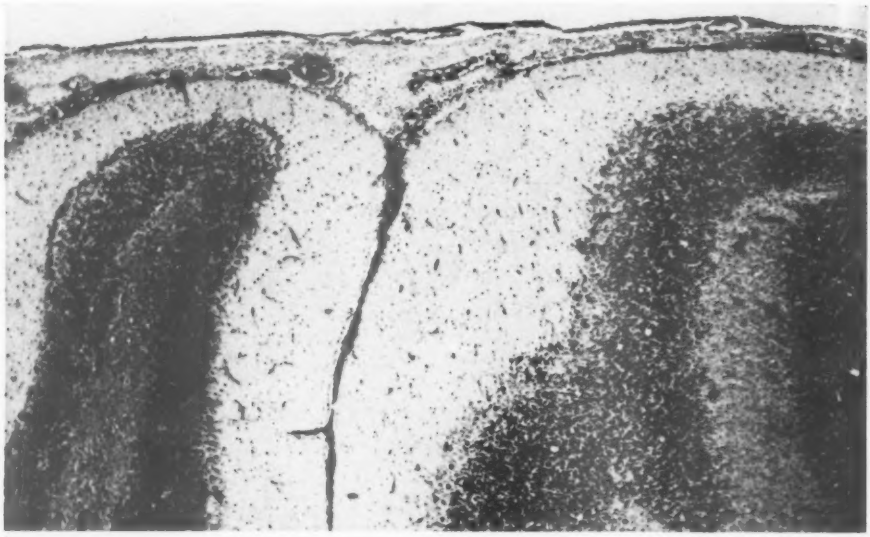


9



10

11



12

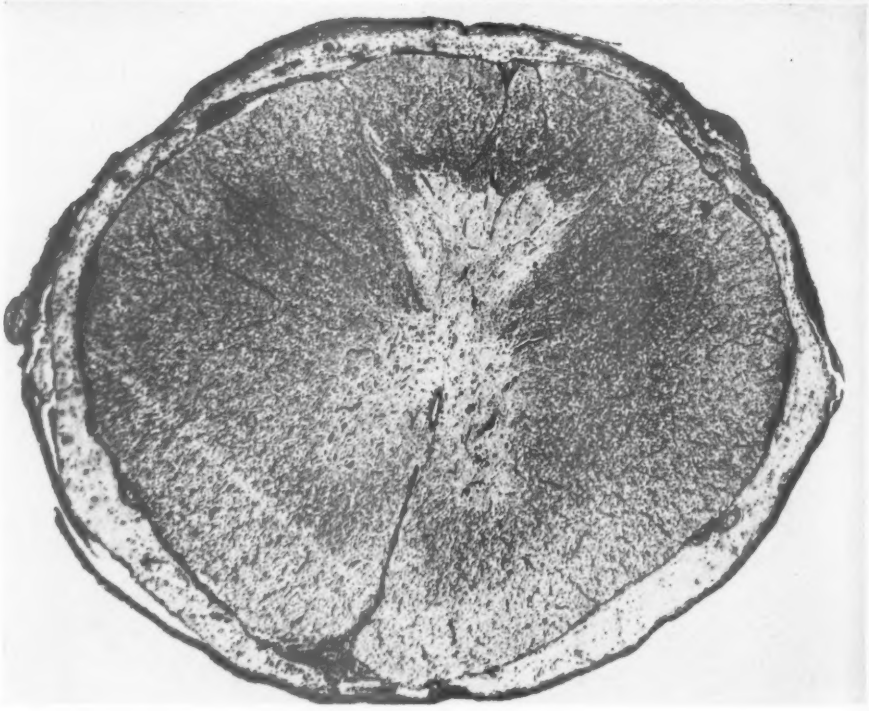
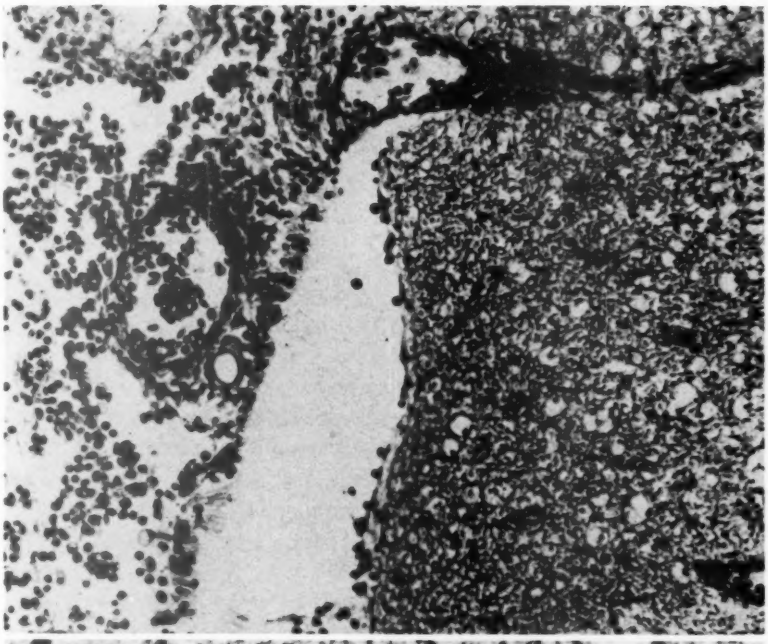
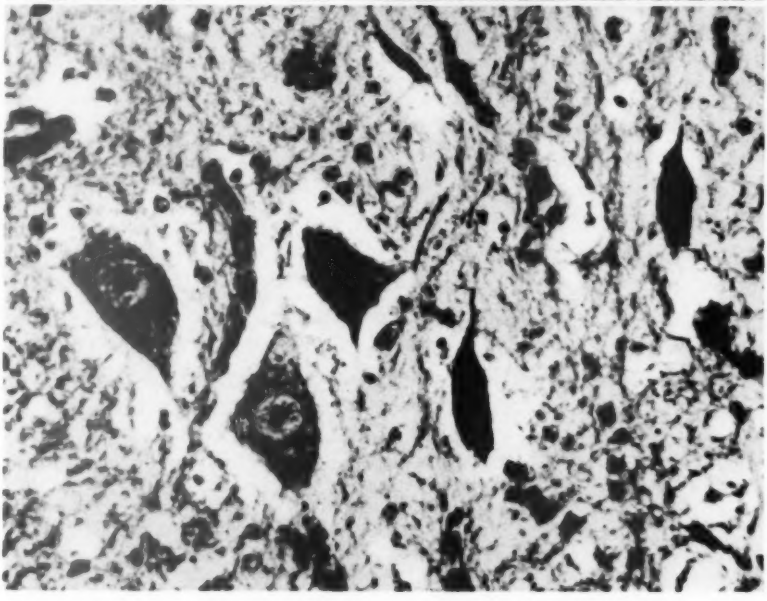


FIG. 11. Cerebellar leptomeningitis. × 45.

FIG. 12. Spinal leptomeningitis. The subarachnoid space distended by fibrinous exudate. × 25.



13



14

FIG. 13. Inflammatory cells in leptomeninges of the medulla oblongata and perivascular cuffing of veins. × 220.

FIG. 14. Spinal cord with pyknosis of motor neurons. × 400.

MORPHOLOGIC CHANGES IN RABBITS FOLLOWING THE INTRAVENOUS ADMINISTRATION OF MENINGOCOCCAL TOXIN

I. The Effects Produced in Young and in Mature Animals by a Single Injection *

Joel G. Brunson, M.D.†; Charles N. Gamble, B.A., and Lewis Thomas, M.D.‡
From the Department of Pathology (Dr. Brunson and Mr. Gamble) and the Pediatric Research Laboratories of the Heart Hospital (Dr. Thomas), University of Minnesota Medical School, Minneapolis 14, Minn.

The occurrence of morphologic changes in animals following the administration of various types of gram-negative bacterial endotoxins and bacterial filtrates has received considerable attention since the description of the Shwartzman phenomenon in 1928.[1] Most studies have been concerned with the changes which are produced by direct injection of toxin or filtrate into an organ or tissue followed at an appropriate interval of time by an intravenous injection of the same or different material. Comparatively little attention has been given to the changes which may occur following a single intravenous injection of bacterial filtrate.

Apitz,[2] in 1934, reported that such an injection in pregnant rabbits resulted in bilateral renal cortical necrosis in some of the animals. He also reported[3] that a single injection of *Escherichia coli* filtrate in non-pregnant animals resulted in areas of necrosis in the heart, liver, and spleen and in pulmonary edema.

Gerber,[4] in 1936, described certain alterations following the intravenous administration of meningococcal and typhoidal filtrates to rabbits. In a group of 13 animals of unstated weights which were given only one injection of filtrate, venous thrombi were noted in the lungs and bone marrow in 3 cases, in the liver in 2 cases, in the intestine in one case, and in the spleen in 6 cases. One animal also showed the presence of circumscribed unilateral necrosis of the adrenal gland. No renal lesions were reported. The author stated also that "degenerative changes" were present in the heart, liver, and spleen, but did not specify the number of instances in which these occurred.

* This study was supported by grants from the Minnesota Heart Association, the American Heart Association, and the Youngstown Area Heart Association. The second and third papers of this series will appear in the July–August issue.

Received for publication, November 2, 1954.

† Fellow of the American Cancer Society.

‡ Now at Department of Pathology, New York University–Bellevue Medical Center, New York, N.Y.

In 1943 Morgan[5] described certain changes following the intravenous injection of a somatic antigen derived from *Eberthella typhosa*. Ten rabbits of unstated weights were given a single injection of 0.5 mg. and the animals died or were killed ½ to 24 hours later. Occasional areas of myocardial necrosis, pulmonary hemorrhages, "hyalinization of liver cells," and "degenerative changes" in the adrenal cortical cells were noted. The author also stated that there was a marked increase in the number of polymorphonuclear leukocytes in the spleen, occasional splenic thrombi, and that the kidneys were congested.

Thomas and Good,[6] in 1952, in a study of the lethal effects of intravenous injections of meningococcal toxin, noted that 2 rabbits of a series of 265 showed "mild bilateral renal cortical necrosis" after a single injection. They suggested that these 2 animals "may have had undetected systemic infections prior to the injection of toxin." It has been noted recently also that a single intravenous injection of endotoxin in rabbits previously given thorotrast or trypan blue,[7] colloidal iron,[8] or cortisone[9] will result in bilateral renal cortical necrosis in a majority of the animals. In the reports dealing with these procedures it was postulated that these substances in some way inhibited or "blocked" the action of the reticulo-endothelial system so that the animal was unable to detoxify or remove from the circulation endotoxin which was subsequently injected. Since two appropriately spaced intravenous injections of meningococcal toxin regularly produce renal cortical necrosis in small rabbits (the generalized Shwartzman phenomenon), it was further postulated that the first injection of endotoxin might have a similar effect on the reticulo-endothelial system.[6]

These accounts appear to constitute the pertinent reports concerning the changes in rabbits following a single intravenous injection of endotoxin and other bacterial filtrates. It seemed of some importance, therefore, to study these changes in more detail. The present paper deals with the morphologic changes which occur following a single intravenous injection of meningococcal toxin. Immature and mature rabbits were used in order to ascertain whether differences in reactivity exist between the two groups.

Materials and Methods

Hybrid albino rabbits of both sexes were used. Group 1 consisted of 50 animals weighing 1 to 1.5 kg., and group 2 consisted of 11 animals weighing 4 to 4.5 kg. They were fed Purina rabbit pellets and had free access to water.

Meningococcal toxin was used throughout the experiments. Details of its preparation have been reported elsewhere.[6] The toxin was diluted with sterile, pyrogen-free, isotonic, saline solution, and injected into the marginal ear vein in a volume of 2 cc. The dilutions used will be indicated in the text.

Following death or sacrifice of the animals, routine post-mortem examinations were done and the tissues were fixed in 10 per cent neutral formalin. Sections were taken from the lungs, heart, kidneys, liver, spleen, pancreas, adrenal glands, and skeletal muscle. The heart was divided after fixation by splitting the interventricular septum, embedding the right half, and further sectioning the left half and the left ventricle into two or three other blocks depending on the size of the heart. This method of cutting showed the entire extent of the septum, ventricles, and valves. In many instances multiple sections were cut from blocks at different levels. Occasionally an entire block was sectioned serially and every fourth section mounted and stained. Hematoxylin and eosin were used routinely and many additional sections were stained by the periodic acid-Schiff method. Selected sections from the spleens and kidneys were stained with toluidine blue, crystal violet, phosphotungstic acid hematoxylin, and van Gieson's stain.

Results

Of the 50 animals comprising the group of small rabbits, 18, or 36 per cent, died following the injection of toxin and the remainder were killed at various intervals. Of the deaths, 16 (89 per cent) occurred prior to 24 hours following administration of the toxin. Of the group of 11 large rabbits, 8 (73 per cent) died within 24 hours following the injection of toxin, one died at 48 hours, and the remaining 2 were killed at that time.

The gross pathologic changes in both groups of animals were usually confined to the lungs and heart. Hemorrhages, usually subpleural, were noted frequently in the lungs. In the heart it sometimes was possible to see irregular areas of yellowish white discoloration in the myocardium, predominantly in the septum. Hemorrhages were noted also in the myocardium in several instances. One animal in each group showed bilateral renal cortical necrosis.

Microscopically, the most consistent changes were noted in the heart. In the animals from each group extensive changes involving the intramural coronary arteries were observed. These consisted of apparent edema and vacuolization of the intimal and medial tunics of the vessels with, at times, a disruption of the endothelial lining so

that it projected into the lumen of the vessel (Fig. 1). These alterations appeared to represent a very early change and were present in those animals which died 2 to 4 hours after the administration of toxin.

In the small animals, in particular, extensive myocardial alterations consisting of areas of hemorrhage, muscle necrosis, calcification of the muscle fibers, and varying degrees of cellular reaction about the necrotic areas were observed. Calcification was more commonly seen in sections from the interventricular septum. In some cases entire segments of muscle appeared calcified, with little evident cellular reaction (Fig. 2). Occasionally a similar change occurred in the intramural coronary arteries. In 2 animals allowed to live for 8 and 9 days following the injection of toxin, segments of vessel walls appeared calcified and showed a surrounding mononuclear cellular reaction.

Areas of hemorrhage and of necrosis of muscle were observed frequently. The latter were often extensive and involved large sections of the myocardium, with a marked mononuclear cellular reaction. In other cases the lesion consisted of small, discrete areas of necrosis and cellularity. In many cases intravalvular hemorrhages were present (Fig. 6) and an occasional valve showed areas suggestive of increased cellularity, but this change was difficult to evaluate.

In the large animals similar lesions of the coronary arteries were seen, but the areas of muscle necrosis and hemorrhage in the myocardium were less extensive. In most cases no significant degree of cellular reaction was noted about the areas of necrotic muscle, the fibers appearing waxy and homogeneous. No areas of calcification were noted in the hearts from this group and in only one case was an intravalvular hemorrhage observed. Hyalinized scars were noted in the myocardium in several of these animals. These probably represented the end stage of damage sustained at some previous time.

The myocardial and valvular changes which have been described occurred in 56 per cent of the small animals, and similar but less extensive changes were noted in 45 per cent of the large animals (Table I).

A recent report[10] described the occurrence of fibrinoid in the coronary arteries and heart valves of small rabbits given a single intravenous injection of endotoxin following systemic infection with group A hemolytic streptococci. Similar material was observed in the hearts of some animals from both groups in this series, appearing as an eosinophilic homogeneous layer beneath the endothelium of the coronary arteries and within the substance of the heart valves. When

stained by the periodic acid-Schiff method it appeared dark purple-red. In the hearts from the small animals it was detected after intensive search through many sections in only 6 cases (12 per cent), but in the hearts from the large animals it was found readily in 8 of the 11 cases (73 per cent). The incidence of this material in the hearts of

TABLE I

Changes Following a Single Intravenous Injection of Meningococcal Toxin

Group	Number of rabbits	Dilution of toxin	Cardiac lesions	Fibrinoid in heart	Renal lesions	Splenic changes	Liver thrombi and necrosis	Pulmonary thrombi
Small rabbits	5	1:20	3	2	1	2	2	2
	5	1:60	3	0	0	0	0	0
	40	1:80	22	4	1	3	6	6
Large rabbits	11	1:80	5	8	2	8	4	6

the small animals is quite low, but in the paper which follows[11] it will be shown that a much higher incidence of coronary arterial and valvular fibrinoid occurs in small rabbits after two intravenous injections of meningococcal toxin.

In the hearts from the large animals, many of the vessels appeared heavily invested with fibrinoid, which seemed to involve the entire media in some cases (Fig. 3). In other instances it was present in the very small intramural coronary arteries and appeared as a hyaline, smooth mass which pushed over into the arterial lumen in a fashion simulating that seen in some cases of thrombotic thrombocytopenic purpura (Fig. 5). It is of interest that this material was present in the larger animals even though most of them died within 18 to 24 hours following the injection of toxin. In the hearts from this group 4 cases showed, in addition, the presence of granular thrombotic material in the intramural coronary arteries; the morphologic appearance of this material suggested that it might be composed of platelets.

In one animal from each group, as noted previously, bilateral renal cortical necrosis was observed. Microscopically, in an additional animal in each group an eosinophilic, slightly granular material containing enmeshed red blood cells was observed in the glomerular capillaries. In neither case was there associated tubular necrosis. The deposition of this material, however, may have been an early stage in the development of cortical necrosis, since the presence of similar material in the glomerular capillary loops has been noted to precede

the development of gross cortical necrosis.[6] No significant alterations were noted in sections from the remaining kidneys.

Sections from the spleens showed accumulations of material in the sinusoids which was homogeneous and distinctly eosinophilic. Occasionally it appeared slightly vacuolated and contained some nuclear débris. It was present in the spleens from the large animals in much greater quantity than in those from the small rabbits, and in 2 cases in the former group was associated with multiple areas of infarction (Fig. 4). The material was noted in the spleens from the small animals in only 5 instances (10 per cent) but was present in 8 of the 11 large rabbits (73 per cent).

Sections of the spleens and kidneys which contained this material were subjected to special staining procedures with the following results: With toluidine blue, a homogeneous blue color; with crystal violet, a purple to reddish purple; with phosphotungstic acid hematoxylin, a homogeneous purple with occasional fine fibrils and an orange tint; with van Gieson's stain, a yellow color; and with the periodic acid-Schiff method, a deep purple-red color. These staining reactions are similar to those described by Altshuler and Angevine[12] as typical for fibrinoid. The development of this material is discussed in detail in the papers which follow.[11,13]

In the lungs, as mentioned previously, hemorrhages were noted frequently. Microscopically, these varied from small, localized areas to rather extensive ones involving considerable portions of the pulmonary parenchyma. Thrombi or emboli were present also in some sections and usually were found in large branches of the pulmonary arteries. They usually were homogeneous and distinctly eosinophilic, but occasionally were noted to contain nuclear débris. At times they appeared completely covered by endothelium. Some of these thrombi were Schiff-positive, others were not. They were observed in 16 per cent of the lungs from the small rabbits and in 55 per cent of the lungs from the large rabbits.

Similar thrombi were noted in the liver, where they appeared to lie in the lumina of efferent veins. Their presence frequently was associated with areas of necrosis which were sometimes hemorrhagic and sometimes pale. The necrotic areas were more extensive in the liver sections from the large animals, in spite of the fact that these rabbits died at shorter intervals following the injection of toxin. Usually the foci of necrosis were surrounded by a cellular zone, but this feature was lacking in most of the sections from the large animals. These thrombi, or thrombi and necrosis, were noted in 16 per cent of the

liver sections from the small rabbits and in 36 per cent of those from the large animals.

One section of the pancreas from a large rabbit showed an area of hemorrhage and necrosis, but no changes in this organ were noted in other animals. Sections from the adrenal glands and skeletal muscle in both groups showed no changes.

Discussion

The results of these experiments indicate that significant changes may occur in rabbits following a single intravenous injection of endotoxin. Some of these changes appear to depend upon the age of the animal, and certain striking differences exist between the reactions of young and of mature rabbits to such an injection.

In the group of small animals, for example, approximately one third died within 24 hours following the injection, while in the group of large animals about two thirds died within the same period. This difference in susceptibility to the lethal effect of endotoxin and the prevention of this lethal effect by pretreatment with cortisone have been reported recently.[14,15]

The morphologic changes in both groups of animals, with one major exception, appear comparable. Both showed diffuse coronary arterial damage which, from the results of other experiments reported in the following paper,[11] appears to be an important factor in the localization of fibrinoid in the heart. The incidence of myocardial damage in both groups is similar, although the cellular reaction about necrotic cardiac muscle was definitely less in the hearts from the large animals. The same difference existed with respect to necrotic areas in the liver. These differences are probably related to the shorter survival time of the large animals following the injection of toxin.

The myocardial lesions, in many respects, resemble those produced by ischemia. It is not unreasonable, in view of the fact that thrombi were observed in the lungs and liver, and in 4 cases in the coronary arteries, to assume that occlusion of the small coronary arteries may have been an important factor in initiating the lesions. However, other factors such as a direct toxic effect of the injected material, or spasm of the coronary arteries, should be considered. It is possible that all three of these factors may be involved in the development of the myocardial changes.

The most striking morphologic difference between the two groups of animals, and one which cannot be explained on the basis of survival time, is the occurrence of fibrinoid in the coronary arteries and heart

valves, and the presence of similar material in the splenic sinusoids in a majority of the mature rabbits. It is of interest that, in 4 animals, material with similar morphologic and tinctorial features was present in the renal glomerular capillaries; in 2 cases, associated with cortical necrosis. As has been suggested,[6] the occurrence of renal cortical necrosis following a single injection of endotoxin may be related to systemic infection at the time of injection. It is conceivable that undetected infection or the results of previous infections may have been a factor in the production of the lesions in the kidneys, spleens, and hearts of the mature animals.

Summary and Conclusions

The effects of a single intravenous injection of meningococcal endotoxin in 50 albino rabbits weighing 1 to 1.5 kg. and in 11 albino rabbits weighing 4 to 4.5 kg. were studied. Of the small animals, 36 per cent died (89 per cent of the deaths occurring prior to 24 hours) and the remainder were killed at varying intervals following the administration of the toxin. Of the large animals, 73 per cent died within 24 hours following the injection of toxin; one other died 48 hours after the injection, and the remaining 2 were killed at that time.

In 56 per cent of the small animals, structural changes were observed in the heart. These consisted of myocardial and valvular hemorrhages, muscle necrosis and cellular reaction, and calcification of the muscle fibers. Similar changes were observed in 45 per cent of the hearts from the large animals, but there was less cellular reaction about the necrotic foci and no areas of calcification were noted. In both groups extensive coronary arterial damage was observed. This was characterized by edema and vacuolization of the intima and media and a disruption of the endothelial lining so that it projected into the lumen of the vessel.

Fibrinoid was found beneath the endothelium of the coronary arteries and within the heart valves in 12 per cent of the small animals and in 73 per cent of the large rabbits. Similar material, with the morphologic and tinctorial features of fibrinoid, was noted in the splenic sinusoids in 10 per cent of the small rabbits and in 73 per cent of the large ones. The same type of material was observed in the renal glomerular capillary loops in 2 animals in each group. In one animal in each group its presence was associated with bilateral cortical necrosis, resembling in all respects that seen in the generalized Shwartzman phenomenon.

Pulmonary hemorrhages and thrombi within branches of the

pulmonary arteries were noted in 16 per cent of the small animals and in 55 per cent of the large ones. Similar thrombi, often with associated areas of necrosis, were observed in the liver in 16 per cent of the small animals and in 36 per cent of the large ones.

It is concluded that a single intravenous injection of endotoxin is followed by a significant mortality rate and definite structural changes in both immature and mature rabbits. Mature rabbits, however, appear much more reactive to a single injection both from the standpoint of its lethal effect and the morphologic changes produced.

The photographs were made by Mr. Henry Morris.

REFERENCES

1. Shwartzman, G. Studies on *Bacillus typhosus* toxic substances. I. Phenomenon of local skin reactivity to *B. typhosus* culture filtrate. *J. Exper. Med.*, 1928, 48, 247–268.
2. Apitz, K. Die Wirkung bakterieller Kulturfiltrate nach Umstimmung des gesamten Endothels beim Kaninchen. *Virchows Arch. f. path. Anat.*, 1934, 293, 1–33.
3. Apitz, K. A study of the generalized Shwartzman phenomenon. *J. Immunol.*, 1935, 29, 255–266.
4. Gerber, I. E. The Shwartzman phenomenon in the kidneys of rabbits. Observations on effects of intravenous administration of bacterial filtrates. *Arch. Path.*, 1936, 21, 776–796.
5. Morgan, H. R. Pathologic changes produced in rabbits by a toxic somatic antigen derived from *Eberthella typhosa*. *Am. J. Path.*, 1943, 19, 135–145.
6. Thomas, L., and Good, R. A. Studies on the generalized Shwartzman reaction. I. General observations concerning the phenomenon. *J. Exper. Med.*, 1952, 96, 605–624.
7. Good, R. A., and Thomas, L. Studies on the generalized Shwartzman reaction. II. The production of bilateral cortical necrosis of the kidneys by a single injection of bacterial toxin in rabbits previously treated with thorotrast or trypan blue. *J. Exper. Med.*, 1952, 96, 625–641.
8. Smith, R. T.; Thomas, L., and Good, R. A. Generalized Shwartzman reaction. V. Intravenous injection of colloidal iron or carbon on response of rabbits to meningococcal toxin. *Proc. Soc. Exper. Biol. & Med.*, 1953, 82, 712–715.
9. Thomas, L., and Good, R. A. The effect of cortisone on the Shwartzman reaction. The production of lesions resembling the dermal and generalized Shwartzman reactions by a single injection of bacterial toxin in cortisone-treated rabbits. *J. Exper. Med.*, 1952, 95, 409–428.
10. Thomas, L.; Denny, F. W., Jr., and Floyd, J. Studies on the generalized Shwartzman reaction. III. Lesions of the myocardium and coronary arteries accompanying the reaction in rabbits prepared by infection with group A streptococci. *J. Exper. Med.*, 1953, 97, 751–766.
11. Brunson, J. G.; Thomas, L., and Gamble, C. N. Morphologic changes in rabbits following the intravenous administration of meningococcal toxin. II. Two appropriately spaced injections; the rôle of fibrinoid in the generalized Shwartzman reaction. *Am. J. Path.* (In press.)

12. Altshuler, C. H., and Angevine, D. M. Histochemical studies on the pathogenesis of fibrinoid. *Am. J. Path.*, 1949, **25**, 1061–1077.

13. Brunson, J. G.; Davis, R. L., and Thomas, L. Morphologic changes in rabbits following the intravenous administration of meningococcal toxin. III. The effects produced by endotoxin in association with certain high molecular weight acidic polymers. *Am. J. Path.* (In press.)

14. Smith, R. T., and Thomas, L. Influence of age upon response to meningococcal endotoxin in rabbits. *Proc. Soc. Exper. Biol. & Med.*, 1954, **86**, 806–809.

15. Thomas, L., and Smith, R. T. Effect of cortisone on response to endotoxin in mature rabbits. *Proc. Soc. Exper. Biol. & Med.*, 1954, **86**, 810–813.

LEGENDS FOR FIGURES

All sections were stained with hematoxylin and eosin.

FIG. 1. Coronary artery showing extensive intimal and medial vacuolization with partial disruption of the endothelial lining. From a small rabbit which died less than 24 hours after an intravenous injection of meningococcal toxin. × 250.

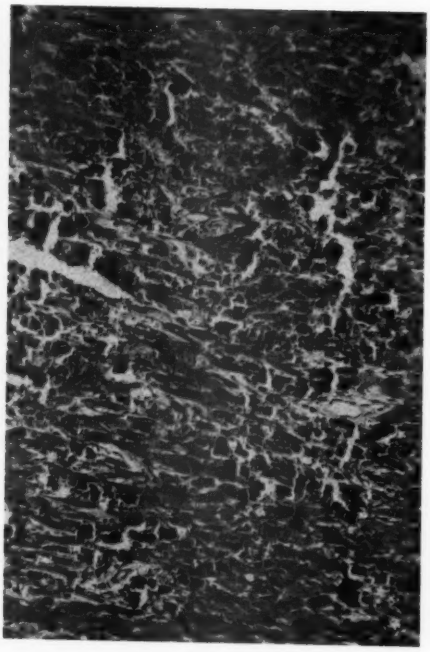
FIG. 2. Section of the myocardium from a small rabbit killed 48 hours after the injection of meningococcal toxin. There is extensive calcification of muscle with little cellular reaction. × 100.

FIG. 3. Pericardial coronary artery with fibrinoid replacement of most of the media. There is absence of cellular reaction. From a large animal killed 48 hours after the injection of toxin. × 450.

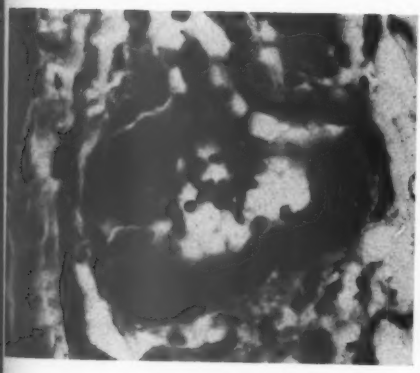
FIG. 4. Section of the spleen from a large rabbit which died 48 hours after the injection of toxin. Area of hemorrhage and necrosis surrounds an accumulation of amorphous material in the sinusoids. × 65.

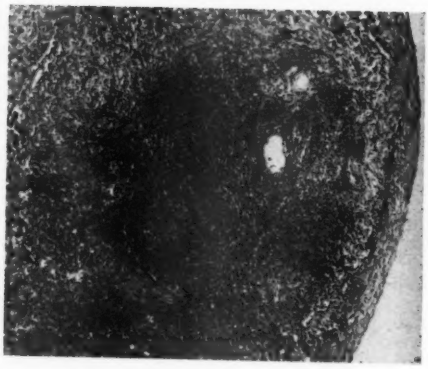
FIG. 5. Intramural coronary artery showing a smooth hyaline subendothelial "thrombus" which projects into the lumen of the vessel. From a large rabbit which died 48 hours after an injection of endotoxin. × 250.

FIG. 6. Mitral valve from a small rabbit killed 72 hours after an injection of meningococcal toxin, showing extensive hemorrhage. × 100.

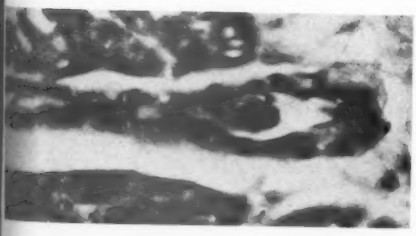


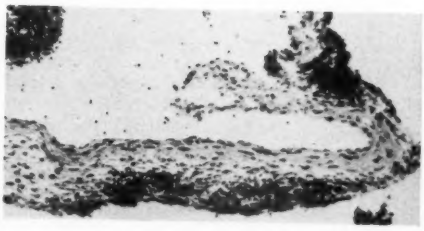
2





4





6

LESIONS OF SKELETAL MUSCLE IN LEPTOSPIROSIS

Review of Reports and an Experimental Study *

Alan R. Laurain, M.D.†

From the Oakes Research Laboratory, St. Luke's Hospital, Denver, Colo.

In recent years, physicians and veterinarians have become increasingly aware of the high incidence of leptospiral infection of man and animals in this country. Several excellent reviews[1-5] and a monograph[6] have been published and repetition is not in order. In an extensive search of the literature, mention of pathologic changes in skeletal muscle was found in only 10 instances of leptospirosis.[7-16] Most of these were human infections. This is in striking contrast to the numerous reports stressing the clinical importance of myalgia and, frequently, of muscle tenderness in leptospiral diseases. These symptoms and signs commonly involve the calf and lower lumbar muscles in *Leptospira icterohaemorrhagiae* and *Lept. canicola* infections. A similar distribution is noted in leptospiral diseases which have more recently attracted attention in the United States: *Lept. pomona* (aseptic meningitis, swineherd's disease)[17-20]; *Lept. grippo-typhosa* (field fever)[21]; and *Lept. autumnalis* (mud fever, Fort Bragg fever).[22,23] A standard textbook[24] states that the muscle lesion of Weil's disease is specific, and is caused by lodgment of the organisms within muscle fibers. From the character and distribution of the lesions, this pathogenetic concept is a logical assumption, but it has not been proved. In experimental studies,[25-28] histologic examination was infrequent and did not include skeletal muscle. Consequently, a study of natural and experimental leptospirosis in small animals was undertaken with particular reference to skeletal muscle. To attain this end, dog and rat tissues were examined histologically and isolation of a virulent Leptospira attempted. Hamsters and guinea-pigs were studied culturally and histologically at various times after injection of a strain of *Lept. canicola*‡ lethal for hamsters in 3 to 5 days.

Materials and Methods

Before beginning this study, I examined a strain of *Lept. icterohaemorrhagiae*§ in whole blood and in organ-Ringer's emulsion to

* Received for publication, August 14, 1954.

† Now at Bristol Memorial Hospital, Bristol, Tenn.

‡ Korgan strain supplied by Dr. Ernst L. Biberstein, New York State Veterinary College (Cornell), Department of Pathology and Bacteriology, Ithaca, New York.

§ Supplied by the American Type Culture Collection, Washington, D.C.

become familiar with the characteristics of the organisms under conditions anticipating those encountered in this investigation. Confusion with the so-called pseudo-spirochaetes[29] is hardly possible when one has seen real organisms. Dark-field preparations of kidney emulsion and blood were examined from all animals. Serologic studies were not done. All cultures were placed on Chang's semisolid medium[30] prepared with fresh horse serum, and on 12 per cent unbuffered fresh rabbit serum in distilled water with 6 cc. of 2 per cent agar per 100 ml. of medium. Enough whole rabbit blood was added to impart a faint pink color. The media were fractionally sterilized at 56° C. for 2 hours on 3 successive days and checked for sterility before use. The final pH was 7.6 in each medium.

Dogs and Rats

Four dogs which died of clinically typical leptospirosis were studied in addition to a control dog which died of high intestinal obstruction. All had received intensive antibiotic therapy, chiefly penicillin and aureomycin. Cultures were taken from contaminated kidney tissue and blood. Kidney emulsion from 2 dogs was injected intraperitoneally into young hamsters.

Eleven wild city rats were killed with chloroform and necropsied immediately. The thoraco-abdominal hair was shaven, the skin thoroughly washed with soap and water, and then swabbed with iodine-alcohol. Cultures of liver and kidney emulsion were planted on separate tubes of each medium using aseptic precautions. Pooled kidney emulsion of 2 rats obtained on the same day was injected intraperitoneally into young hamsters.

Hamsters and Guinea-Pigs

Control and experimental animals were fed a routine laboratory diet and given only leafy green vegetables as a source of water. The group cages were separated by a distance of ten feet.

A group of 18 hamsters, 3 to 4 weeks old, were injected intraperitoneally with 0.5 ml. of infected hamster kidney-Ringer's emulsion containing 5 to 10 *Lept. canicola* per oil immersion dark-field. One animal each was chloroformed at 1, 2, 60, and 72 hours after injection. At 3, 6, 12, 24, and 48 hours, animals were sacrificed 2 at a time. Three hamsters were found dead and necropsied at 96 hours. One died and was necropsied at 120 hours. At necropsy, cultures were taken from intracardial blood and from kidney using the technique outlined. Pooled skeletal muscle from a group of dead or moribund

hamsters used in maintaining the strain was examined for the presence of muscle lesions. The control group consisted of 3 untreated hamsters and 7 injected intraperitoneally with 0.5 cc. of kidney-Ringer's emulsion from a normal hamster. One control animal each was sacrificed at 3, 24, and 48 hours after injection and 2 each at 6 and 12 hours.

A program similar to that used in the hamsters was carried out with 12 guinea-pigs, varying from 3 days to 3 weeks old. Three normal controls and 12 injection controls were used.

At necropsy, portions of liver, kidney, heart, lungs, pectoralis major, and of longissimus dorsi and gastrocnemius muscles were routinely fixed in 10 per cent neutral formalin. These were blocked in paraffin, cut at 6 μ, and stained with hematoxylin and eosin. Selected blocks of kidney, liver, skeletal muscle, and myocardium were cut at 10 μ and stained by Dieterle's[31] and Steiner's[32] methods. Levaditi stains also were employed on some of the tissue. All silver stains were controlled using dog kidney known to contain leptospirae (Fig. 1). This tissue was initially controlled with liver from a case of congenital syphilis. When the control was negative, that lot of slides was discarded. In kidney the possibility of confusing elastic fibers, cytoplasmic membranes, and other artifacts with leptospirae is minimal. Therefore, that organ was used exclusively for diagnostic purposes. The following criteria were met before accepting a kidney as positive for leptospirae: intact cell walls and nuclear membranes; suspected structure to be of uniform diameter and entirely within the cytoplasm, without touching cell boundaries or nuclear membrane; at least three such structures to be observed in one section.

Results

In spite of aseptic precautions, over three fourths of the organ cultures were contaminated. In such instances neither Chang's nor the rabbit serum medium succeeded in supporting leptospiral growth, although simultaneous blood cultures from the same animals frequently were positive for both. As detailed in the discussion, the muscle reactions are indicated numerically by histologic type. Briefly, these are as follows:

I. Focal damage to isolated fibers
II. Diffuse or focal interstitial inflammation
III. Diffuse Zenker's degeneration
IV. Diffuse vacuolar degeneration
V. Diffuse atrophy

Dogs and Rats

Two dogs had gross changes typical of the uremic form of leptospirosis, with bulging, yellow, renal cortices and relative sparing of the medulla. There were multiple, focal, pulmonary hemorrhages, gastrointestinal hemorrhage, and varying degrees of parenchymal hemorrhage into other organs. The other two were markedly icteric and showed a narrow yellow line at the corticomedullary junction. Hemorrhagic phenomena, though present, were less striking than in the renal form. The livers of all dogs were severely congested but otherwise grossly normal. The extrahepatic bile ducts and skeletal muscles also were grossly normal in all. All dark-field preparations and cultures were negative. The injected hamsters remained healthy. The control dog also showed a yellow corticomedullary junction in the kidneys, but no hemorrhagic phenomena.

Microscopically, interstitial nephritis and tubular degeneration corresponded in degree to the gross appearance. Figure 2 illustrates the severe renal form. The icteric dogs had less severe renal changes resembling those found in rats. Organisms were seen in the kidneys of an icteric and a non-icteric dog. Very rare foci of necrosis were seen in the livers of 3 animals. The icteric dog with proved leptospirosis showed a characteristic type 1 muscle reaction (Fig. 3) which was obviously quite similar to the lesion illustrated in Figure 4 from a proved human case of Weil's disease.* The other icteric dog had focal type 1 lesions in an earlier stage with beginning débridement by macrophages (Fig. 13). The dog with proved renal leptospirosis had multiple, focal, muscle lesions with very severe disintegration of the sarcoplasm and associated marked interstitial edema (Fig. 12). This was classified as type 1 because of the focal involvement. A single fiber in this animal showed changes like those in Figures 3 and 4. The other animal showed no muscle lesions in multiple blocks. Fatty tubular degeneration and slight interstitial calcification at the renal corticomedullary junction was seen in the control dog and probably resulted from metabolic alkalosis. No muscle lesions were seen.

All dark-field preparations and cultures from the rats were negative. Diffuse interstitial myocarditis of unknown cause and liver granulomas due to *Capillaria hepatica* were found in nearly every animal. Less frequent conditions were bronchiectatic lung abscesses and Sarcocystis infestation. One animal had demonstrable trichinosis. Four animals exhibited mild interstitial nephritis (Fig. 5) and hemo-

* From personal slide collection. This case has been reported in the literature.[11]

siderosis of the hepatic portal zones. A type 1 muscle reaction (Fig. 6) was seen in all of these animals, involving rare scattered fibers. Figure 7 from a proved human case of Weil's disease may be seen for comparison. Leptospiras were found in one rat kidney (Fig. 8), adjacent to an area of interstitial nephritis. Interstitial inflammatory cells, chiefly eosinophils, were seen around the Trichinella larvae. All other animals which lacked the liver and renal changes also lacked muscle lesions, regardless of the combination of other conditions. None of the animals exhibited muscle fiber damage or cellular reaction to the Sarcocystis organisms, although some were very heavily infested. The injected hamsters remained healthy, but neither of the rats used showed lesions attributable to leptospirosis.

Hamsters and Guinea-Pigs

Grossly, the hamsters appeared normal until 12 hours after inoculation when rare, circular, pulmonary hemorrhages became evident. These increased in number until 60 hours when they reached their maximum (Fig. 9) and then began to decrease. At that time the first positive direct dark-field examinations on both blood and kidney were obtained. Thereafter, leptospirae were often more numerous in the animal blood than in cultures at the peak of the growth curve. Active leptospirae were seen in kidney emulsion at 48 hours. Blood cultures were positive on all animals after 3 hours. The kidneys were congested at 48 hours and intensely hemorrhagic at all later intervals. The skeletal muscles were normal grossly until shortly before death when interstitial hemorrhage was apparent.

Histologically, focal pulmonary alveolar hemorrhages closely paralleled the gross appearance in size and number. Progressively increasing renal lesions appeared at 60 hours, consisting of diffuse interstitial and glomerular hemorrhage, and hemoglobin casts with marked tubular necrosis in the proximal tubules. Therefore, the established criteria for identification of leptospirae could be met only at the 48th and 60th hours although leptospira-like structures increased progressively in the interstices and necrotic tubular cells. A striking feature in the organs studied was the lack of inflammatory cellular reaction to the leptospiral infection. That the animals were capable of cellular reaction was demonstrated by the occurrence of abscesses in several animals in which organ emulsion had inadvertently been injected subcutaneously.

At the third and sixth hours a focal, type II muscle reaction without associated muscle fiber damage was seen. This change was present in

controls necropsied at the same intervals. No inclusion bodies were seen in either group. Later, similar reaction patterns were seen only in association with gross or microscopic interstitial hemorrhage. At the time of death, focal hyalinization of portions of muscle fibers (Fig. 10) often was seen, but again in association with marked interstitial hemorrhage. A characteristic type I reaction was not observed in any of the animals. Leptospira-like structures were seen within interstitial capillaries but not within the muscle fibers themselves.

Two guinea-pigs, 3 days old at the time of injection, succumbed at 146 hours. Two older animals survived without indication of illness and were sacrificed at 11 and 18 days. The other animals were chloroformed at intervals comparable to those for the hamsters. All blood cultures were positive except in the 2 which survived. The dark-field examination of kidney became positive at 72 hours and of blood at 96 hours. Leptospirae were readily demonstrable in the renal tubules at 96 hours and in all others studied at later intervals except in the animal killed at 11 days. All animals except the 2 which survived showed changes grossly similar to, but less extensive than, those in hamsters. These were first recognizable in the lungs at 48 hours, reached a peak at 72 hours, and then decreased. The kidneys bore a striking resemblance to the "flea bitten" kidney of malignant nephrosclerosis. Microscopically, the pulmonary alveolar hemorrhages (Fig. 11) were identical with those in the hamsters. The renal lesions also were similar, but very mild interstitial nephritis was present and the tubular epithelium much better preserved. The animal living 11 days had only mild interstitial nephritis. The animal sacrificed at 18 days had diffuse interstitial nephritis which was similar but less extensive than in the icteric dogs. Kidney emulsion from this animal containing rare, viable leptospirae was injected into a hamster. There was no evidence of leptospirosis on several blood cultures from the hamster or at necropsy 5 days after injection.

Grossly, some of the skeletal muscles of the guinea-pigs showed interstitial hemorrhages. Microscopically, these were associated with focal hyalinization of muscle fibers like that in the hamsters. Characteristic type I reactions were not seen. The skeletal muscles of the controls were normal.

Discussion

It is evident that the ability of skeletal muscle to respond to injury is rather limited[33-35] and similar in many conditions. Various authors[33,34,36,37] have shown that each damaged fiber reacts by formation of a "retraction cap of injury" which histologically has a waxy

appearance like that of Zenker's degeneration. More severely damaged fibers are markedly fragmented and the sarcolemma collapsed. Débridement is begun within 12 hours by invading macrophages and shortly thereafter muscle nuclei begin mitotic and amitotic proliferation to form sarcoblasts. This produces a sarcolemmal tube filled with cells. Often by the second day after injury, regenerating muscle fibers about one fourth the normal size are directed by the sarcolemma into the damaged area, the "peripheral" regeneration. Less frequently, in "terminal" regeneration, growth proceeds from the entire width of the preserved portion of the damaged fibers. All individual injured fibers undergo these changes unless the damage is very extensive, in which case repair by fibrosis is seen. However, there is a wide variation in over-all appearance depending upon the type of noxious agent and the extent of muscle involvement. The muscles involved also vary. With full realization that arbitrary classification of a dynamic pathologic process is inadequate at best, the following general reaction patterns were formulated to facilitate a comparison of leptospiral myopathies with other conditions (Table I).

I. Leptospiral Type. Focal involvement of isolated muscle fibers and parts of fibers with hyalinization, vacuolization, proliferation of muscle nuclei,* invasion by macrophages and occasional neutrophils, but with good preservation of the sarcolemma, confinement of the cellular reaction within the muscle fiber, and repair by regeneration, or occasionally, fibrosis (Figs. 3, 4, 6, 7, 12, and 13).

II. Coxsackie Virus, Rickettsial Type. Diffuse or focal interstitial and/or perivascular infiltration by macrophages, large mononuclear cells, lymphocytes and variable numbers of neutrophils, with or without the muscle fiber involvement designated as type I.

III. Toxemic Type. Diffuse Zenker's hyaline degeneration with repair by fibrosis or fiber regeneration through type I.

IV. Hypothermic Type. Intense diffuse vacuolar degeneration, fragmentation, collapse and disruption of the sarcolemma, and frequent heavy infiltration by neutrophils with repair by diffuse fibrosis or regeneration of surviving fibers through the intermediary of type I.

V. Atrophic-Dystrophic Type. A mixed reaction with focal areas resembling types II and III, pseudo-hypertrophy, regenerative or fibrous repair, and atrophy with replacement by adipose tissue or formation of lymphorrhages.

* The term muscle nuclei refers to proliferating cells within injured muscle fibers which are often called sarcolemma nuclei. The sarcolemma has no nuclei.[45] Furthermore, the identity of the cells in question as muscle nuclei has been shown convincingly in living muscle fibers.[34]

TABLE I

Types of Reaction and Results in Various Myopathic Diseases

Condition	Host	Type of reaction	End results	Author	Remarks
Coxsackie virus Conn. 5, Ohio 1	Mouse	I	Regeneration	Godman *et al.*[33]	
Texas 1, Easton 2	Mouse	II			
Poliomyelitis	Hamster	I(?)	Not stated	Aronson and Shwartzman[38]	Not illustrated; lesions in cortisone treated animals only
Physical injury	Rabbit	I	Regeneration	Clark[36]	
Encephalomyelitis virus	Mouse	II	Calcification & chronic inflammation	Rustigian and Pappenheimer[39]	
Potassium deficiency	Dog	I	Regeneration	Smith *et al.*[40]	Fig. 3, parts C and D, p. 195, ref. 40, indistinguishable from the lesion of Weil's disease
Pneumonia	Human	II	Regeneration	Forbus[37]	Abdominal muscles only
Physico-chemical injury	Tadpole	I	Regeneration	Speidel[34]	Single living fibers studied
Frostbite	Dog	IV	Regeneration	Pirozynski and Webster[41]	
Roentgen rays	Rabbit	IV	No regeneration in 7 days	Lewis[42]	Neutrophilic infiltration
Myasthenia gravis	Human	V	Atrophy	Russell[43]	
Nutritional muscular dystrophy	Rabbit	V	Not stated	Innes and Yevich[44]	
Leptospirosis icterohaemorrhagiae	Human	I	Not stated	Pick[7]	
icterohaemorrhagiae	Human	I	Not stated	Jeghers *et al.*[8]	

icterohaemorrhagiae	Human	I	Regeneration	Sheldon[9]	
icterohaemorrhagiae	Human	III(?)	Not stated	Fialho[10]	Cited by DaCorso[16]
icterohaemorrhagiae	Human	I	Not stated	Cowden *et al.*[11]	
icterohaemorrhagiae	Human	I	Not stated	Sheldon[12]	Antibody demonstrated in muscle lesion
Leptospirosis canicola	Human	I(?)	Not stated	Wolff *et al.*[13]	Not illustrated
canicola	Human	I(?)	Not stated	Turrell and Hamburger[14]	Lesion not evident in illustration
icterohaemorrhagiae	Dog	Interstitial hemorrhage	Not stated	Monlux[15]	
canicola	Dog	Interstitial hemorrhage	Not stated	Monlux[15]	
icterohaemorrhagiae	Dog	I(?)	Not stated	DaCorso[16]	Type III illustrated but description suggests type I
This study					
icterohaemorrhagiae(?)	Dog	I			See Figs. 3 & 13 of this article
icterohaemorrhagiae(?)	Rat	I			See Fig. 6
canicola(?)	Dog	I			See Fig. 12
canicola (Korgan)	Hamster	Interstitial hemorrhage	Focal "retraction caps" at death attributed to interstitial hemorrhage		
canicola (Korgan)	Guinea-pig	Interstitial hemorrhage	Focal "retraction caps" at death attributed to interstitial hemorrhage		See Fig. 10

It is evident from Table I that a type I muscle reaction in man is characteristic of *Lept. icterohaemorrhagiae* infection. It is not specific as shown by its possible occurrence in other conditions. Theoretically, familial periodic paralysis (potassium deficiency) and infectious pleurodynia (Coxsackie virus) might be expected to have a type I lesion, but these could hardly be confused clinically with leptospirosis. Rickettsial infection has been confused with Weil's disease on muscle biopsy,[9] but careful study of several slides should reveal a type II pattern with pronounced vascular changes. The occurrence of a type I lesion in canicola fever has not been demonstrated convincingly. Reports of muscle lesions in the other leptospiral diseases were not found.

Both dogs with proved leptospirosis and another with typical gross and microscopic findings had skeletal muscle lesions. The negative dark-field examinations, cultures, and animal inoculations are not disturbing since penicillin is known to affect leptospirae *in vivo*.[46] Furthermore, because of marked fragmentation, many fields had to be surveyed before finding an intact organism or group of organisms in the kidney sections. Probably the organisms were dead and the dogs died because of cellular damage sustained before therapy was started. In general, the icteric form of canine leptospirosis is caused by *Lept. icterohaemorrhagiae* and the uremic form by *Lept. canicola*, but exceptions are relatively common.[47] Quite probably, the icteric dogs showing type I muscle reactions were infected with the former.

The diagnosis in rats was conclusive in only one instance, but the combination of hemosiderosis and interstitial nephritis made it likely in the unproved cases. Rats harbor only *Lept. icterohaemorrhagiae*.[6] Therefore, the correlation of these findings with a type I lesion and the lack of correlation with any of the many other conditions also support the diagnosis.

Lept. canicola was used for experiments on the guinea-pig and hamster only because a lethal strain of *Lept. icterohaemorrhagiae* could not be isolated or obtained by mail. The former produces severe myalgia in man, presumably because of muscle lesions like those of Weil's disease. Therefore, it was expected that *Lept. canicola* would produce typical lesions in animals. The absence of characteristic lesions was quite surprising. Ever-increasing leptospiremia in both experimental groups should have afforded the organisms ample opportunity for burrowing into the muscle fibers. Indeed, their demonstration in the renal tubular epithelium of the hamsters and guinea-pigs is taken as evidence of their burrowing capability. One may conclude that in guinea-

pigs and hamsters, *Lept. canicola* (Korgan) does not burrow into muscle fibers or produce characteristic muscle lesions. Whether the same holds true for *Lept. icterohaemorrhagiae* and other strains of *Lept. canicola* in these animals remains to be seen. To assume from this study that the muscle lesion of human Weil's disease is not caused by burrowing organisms would be completely fallacious. In fact, recent histo-serologic evidence[12] supports the burrowing concept. A plausible explanation for the lack of a typical muscle reaction in the hamsters and guinea-pigs and its common occurrence in human Weil's disease is the possibility of electrokinetic phenomena which vary with the host and the leptospiral sero-type.[48] Renal hypopotassemia[49] is another possibility, but seems unlikely. However, it is interesting that dogs with leptospirosis often exhibit motor paralysis of the hind legs. Finally, it appears that young guinea-pigs are susceptible to large doses of *Lept. canicola* (Korgan) without showing outward signs of disease. If these observations can be repeated with smaller doses and other strains of *Lept. canicola,* young guinea-pigs may prove to be as valuable as hamsters in the clinical diagnosis of canicola fever.

Summary and Conclusions

Lesions of skeletal muscle identical with those described in human Weil's disease are reported for the first time in naturally infected dogs and wild city rats with leptospirosis. The literature on reported leptospiral myopathies is summarized and findings compared with muscle lesions in other conditions, using a histologic classification based upon general reaction patterns to various noxa. Guinea-pigs and hamsters experimentally infected with *Leptospira canicola* did not develop characteristic muscle lesions. Young guinea-pigs all developed visceral lesions typical of leptospirosis after large doses of *Lept. canicola* (Korgan). While not specific, in the sense of a tubercle containing acid-fast bacilli, the skeletal muscle lesion of Weil's disease (*Lept. icterohaemorrhagiae*) is sufficiently characteristic to be of diagnostic significance in human disease when interpreted in the light of the clinical history. As far as is known, type I reaction (focal damage to isolated fibers) in humans has been reported only in Weil's disease. The presence or absence of these muscle lesions in man and animals infected with leptospirae other than *Lept. icterohaemorrhagiae* remains to be shown.

Acknowledgment is made to the Department of Medical Illustration, University of Colorado Medical School, for Figure 1 and to Mr. I. S. Stephens for all other photomicrographs, and to Drs. L. S. Peavy and J. R. Naylor for dogs from their practices.

REFERENCES

1. Rosenberg, B. L. Canicola fever; review, with report of two new cases. *Am. J. Med.*, 1951, **11**, 75–91.
2. Gordon, M. E. Canicola fever. Report of first case in Connecticut and review of the literature. *New England J. Med.*, 1952, **247**, 708–714.
3. Rosenbaum, H. D. Canicola fever. Case report and review of the literature. *Arch. Int. Med.*, 1946, **78**, 531–543.
4. Ashe, W. F.; Pratt-Thomas, H. R., and Kumpe, C. W. Weil's disease. A complete review of American literature and an abstract of the world literature. Seven case reports. *Medicine*, 1941, **20**, 145–210.
5. Reinhard, K. R. Parasitological reviews—newer knowledge of leptospirosis in the United States. *Exper. Parasitol.*, 1953, **2**, 87–115.
6. Van Thiel, P. H. The Leptospiroses. Universitaire Pers Leiden, Leiden, 1948, 231 pp.
7. Pick, L. Zur pathologischen Anatomie des infektiösen Icterus. *Berl. klin. Wchnschr.*, 1917, **54**, 451–455, 481–484.
8. Jeghers, H. J.; Houghton, J. D., and Foley, J. A. Weil's disease. Report of a case with postmortem observations and review of recent literature. *Arch. Path.*, 1935, **20**, 447–476.
9. Sheldon, W. H. Lesions of muscle in spirochetal jaundice (Weil's disease; spirochetosis icterohemorrhagica). *Arch. Int. Med.*, 1945, **75**, 119–124.
10. Fialho, A. Sôbre dois casos de doença de Weil no Rio de Janeiro. *Arq. de hig. saúde púb., São Paulo*, 1938, **8**, 37–57. (Cited by DaCorso.[16])
11. Cowden, F. E.; Owenby, F. D., and Isham, R. L. Weil's disease—report of four cases emphasizing two adjuncts to early diagnosis. *Am. Pract.*, 1952, **3**, 353–360.
12. Sheldon, W. H. Leptospiral antigen demonstrated by the fluorescent antibody technic in human muscle lesions of *Leptospirosis icterohemorrhagiae*. *Proc. Soc. Exper. Biol. & Med.*, 1953, **84**, 165–170.
13. Wolff, J. W.; Van Dam, R., and Minkenhof, J. E. The first known fatal case of canicola fever. *Lancet*, 1951, **1**, 1100–1102.
14. Turrell, R. C., and Hamburger, M. Canicola fever with meningitis. Report of a case in a human treated with penicillin. *Am. J. Med.*, 1951, **10**, 249–253.
15. Monlux, W. S. The pathology of canine leptospirosis. *Cornell Vet.*, 1948, **38**, 58–69.
16. DaCorso Filho, P. Contribução á anatomia patológica da leptospirose icterohemorrágica no Cão. *Bol. da Soc. Brasil de Med. Vet.*, 1945, **14**, 7–43.
17. Schaeffer, M. Leptospiral meningitis. Investigation of a water-bourne epidemic due to *L. pomona*. *J. Clin. Investigation*, 1951, **30**, 670–671. (Cited by Reinhard.[5])
18. Sutliff, W. D.; Shepard, R., and Dunham, W. B. Acute *Leptospira pomona* arthritis and myocarditis. *Ann. Int. Med.*, 1953, **39**, 134–140.
19. Coffey, J. H.; Dravin, I., and Dine, W. C. Swineherd's disease (aseptic meningitis) due to *Leptospira pomona*. *J. A. M. A.*, 1951, **147**, 949–950.
20. Beeson, P. B., and Hankey, D. D. Leptospiral meningitis. *A. M. A. Arch. Int. Med.*, 1952, **89**, 575–583.
21. Spain, R. S., and Howard, G. T. Leptospirosis due to *Leptospira grippotyphosa*. *J. A. M. A.*, 1952, **150**, 1010–1012.

22. Daniels, W. B., and Grennan, H. A. Pretibial fever, an obscure disease. *J. A. M. A.*, 1943, **122**, 361–365.

23. Gochenour, W. S., Jr.; Smadel, J. E.; Jackson, E. B.; Evans, L. B., and Yager, R. H. Leptospiral etiology of Fort Bragg fever. *Pub. Health Rep.*, 1952, **67**, 811–813.

24. Cecil, R. L., and Loeb, R. F. (eds.) Textbook of Medicine. W. B. Saunders Co., Philadelphia, 1951, ed. 8, p. 376.

25. Randall, R., and Cooper, H. K. The golden hamster (*Cricetus auratus*) as a test animal for the diagnosis of leptospirosis. *Science*, 1944, **100**, 133–134.

26. Larson, C. L. Experimental leptospirosis in hamsters (*Cricetus auratus*). *Pub. Health Rep.*, 1944, **59**, 522–527.

27. Morton, H. E. Susceptibility of Syrian hamsters to leptospirosis. *Proc. Soc. Exper. Biol. & Med.*, 1942, **49**, 566–568.

28. Reinhard, K. R. A clinical-pathological study of experimental leptospirosis of calves. *Am. J. Vet. Sc.*, 1951, **12**, 282–291.

29. Gowen, G. Pseudo-spirochaetes in blood. *Illinois M. J.*, 1946, **89**, 294–296.

30. Chang, S. L. Studies on *Leptospira icterohaemorrhagiae*. I. Two new mediums for growing *L. icterohaemorrhagiae*, *L. canicola*, and *L. biflexor*, and a method for maintaining the virulence of *L. icterohaemorrhagiae* in culture. *J. Infect. Dis.*, 1947, **81**, 28–34.

31. Dieterle, R. R. Method for demonstration of *Spirochaeta pallida* in single microscopic sections. *Arch. Neurol. & Psychiat.*, 1927, **18**, 73–80.

32. Steiner, G., and Steiner, G. New simple silver stain for demonstration of bacteria, spirochetes, and fungi in sections from paraffin-embedded tissue blocks. *J. Lab. & Clin. Med.*, 1944, **29**, 868–871.

33. Godman, G. C.; Bunting, H., and Melnick, J. L. The histopathology of Coxsackie virus infection in mice. I. Morphologic observations with four different viral types. *Am. J. Path.*, 1952, **28**, 223–257.

34. Speidel, C. C. Studies of living muscles. I. Growth, injury and repair of striated muscle, as revealed by prolonged observations of individual fibers in living frog tadpoles. *Am. J. Anat.*, 1937–38, **62**, 179–235.

35. Adams, R. D.; Denny-Brown, D., and Pearson, C. M. Diseases of Muscle. A Study in Pathology. Paul B. Hoeber, Inc., New York, 1953, p. 556.

36. Clark, W. E. L. An experimental study of the regeneration of mammalian striped muscle. *J. Anat.*, 1946, **80**, 24–36.

37. Forbus, W. D. Pathological changes in voluntary muscle. I. Degeneration and regeneration of the rectus abdominis in pneumonia. *Arch. Path.*, 1926, **2**, 318–399; Pathological changes in voluntary muscle. II. Experimental study of degeneration and regeneration of striated muscle with vital stains. *Ibid.*, 1926, **2**, 486–499.

38. Aronson, S. M., and Shwartzman, G. Histopathogenesis of cortisone-altered experimental poliomyelitis. Observations on the Syrian hamster inoculated intracerebrally with strain MEF_1. *Am. J. Path.*, 1953, **29**, 381–399.

39. Rustigian, R., and Pappenheimer, A. M. Myositis in mice following intramuscular injection of viruses of the mouse encephalomyelitis group and of certain other neurotropic viruses. *J. Exper. Med.*, 1949, **89**, 69–92.

40. Smith, S. G.; Black-Schaffer, B., and Lasater, T. E. Potassium deficiency syndrome in the rat and the dog. A description of the muscle changes in the potassium-depleted dog. *Arch. Path.*, 1950, **49**, 185–199.

41. Pirozynski, W. J., and Webster, D. R. Muscle tissue changes in experimental frostbite. *Ann. Surg.*, 1952, **136**, 993–998.

42. Lewis, R. B. Changes in striated muscle following single intense doses of x-rays. *J. Lab. Investigation*, 1954, **3**, 48–55.

43. Russell, D. S. Histological changes in the striped muscles in myasthenia gravis. *J. Path. & Bact.*, 1953, **65**, 279–289.

44. Innes, J. R. M., and Yevich, P. P. So-called nutritional muscular dystrophy as a cause of "paralysis" in rabbits. *Am. J. Path.*, 1954, **30**, 555–565.

45. Maximow, A. A., and Bloom, W. A Textbook of Histology. W. B. Saunders Co., Philadelphia, 1952, ed. 6, p. 148.

46. Chang, S. L. Studies on *Leptospira icterohaemorrhagiae*. II. A critical study of the effect of penicillin on *Leptospira icterohaemorrhagiae in vitro* and in leptospirosis in guinea pigs. *J. Clin. Investigation*, 1946, **25**, 752–760.

47. Jennings, A. R. Postmortem findings in leptospiral infection in the dog. *Vet. Rec.*, 1948, **60**, 272–273.

48. Stavitsky, A. B. Studies on the pathogenesis of leptospirosis. *J. Infect. Dis.*, 1945, **76**, 179–192.

49. Brown, M. R.; Currens, J. H., and Marchand, J. F. Muscular paralysis and electrocardiographic abnormalities resulting from potassium loss in chronic nephritis. *J. A. M. A.*, 1944, **124**, 545–549.

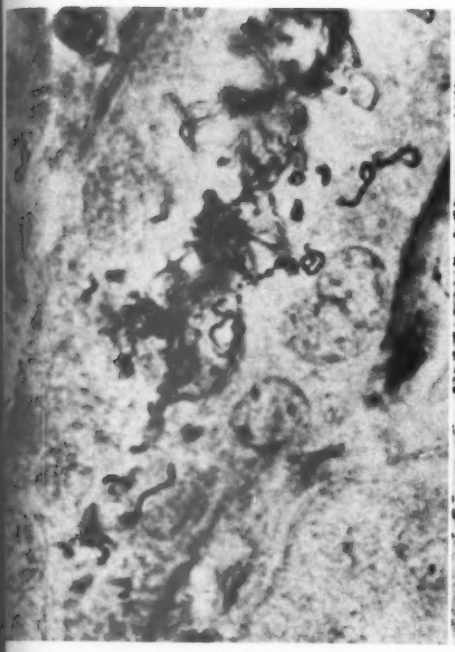
LEGENDS FOR FIGURES

FIG. 1. Kidney of dog with leptospirosis of uremic type. Numerous leptospirae visible. Dieterle's stain. × 1750.

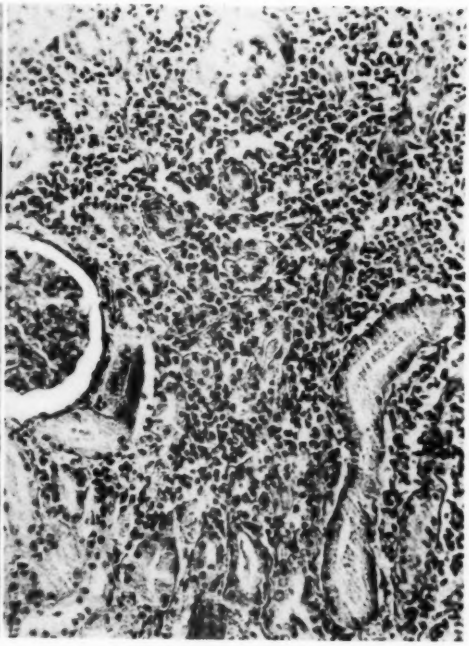
FIG. 2. Kidney of dog with leptospirosis of uremic type. Lymphocytes and plasma cells predominate, but scattered neutrophils also are present. Hematoxylin and eosin stain. × 120.

FIG. 3. Gastrocnemius muscle of dog with proved icteric leptospirosis. Sarcolemmal "tube" formation. Hematoxylin and eosin stain. × 120.

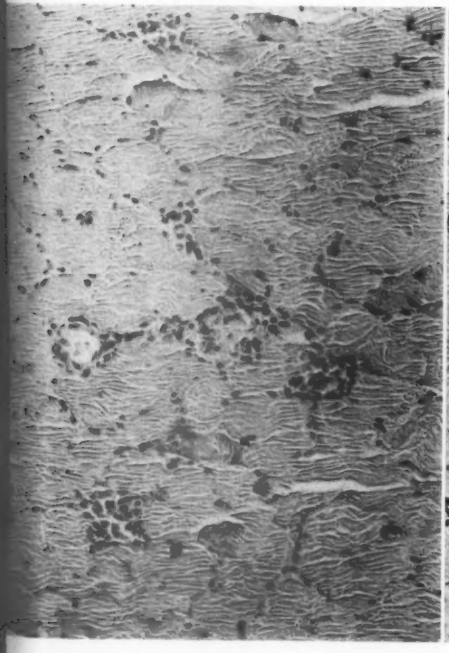
FIG. 4. Human gastrocnemius muscle from serologically proved Weil's disease. Hematoxylin and eosin stain. × 120.



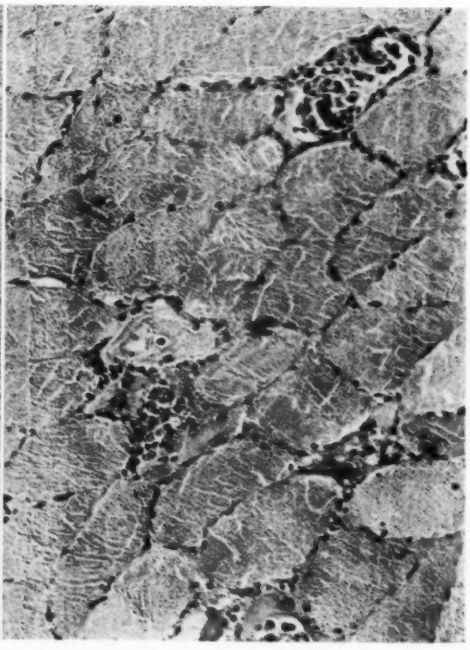
1



2



3



4

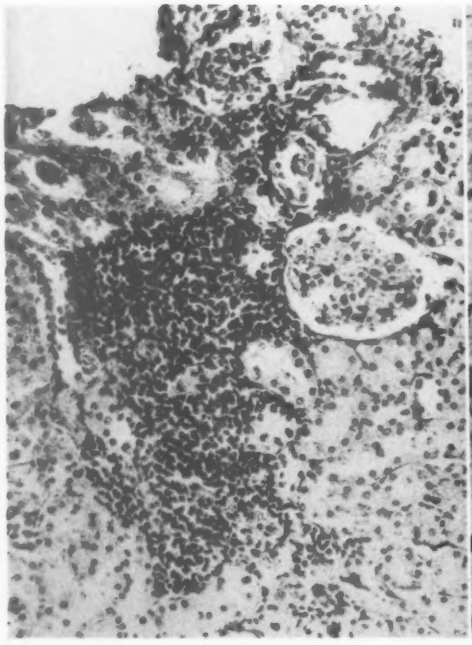
FIG. 5. Kidney of the rat illustrated in Figures 6 and 8. Hematoxylin and eosin stain. × 120.

FIG. 6. Gastrocnemius muscle of the rat illustrated in Figures 5 and 8. Hematoxylin and eosin stain. × 120.

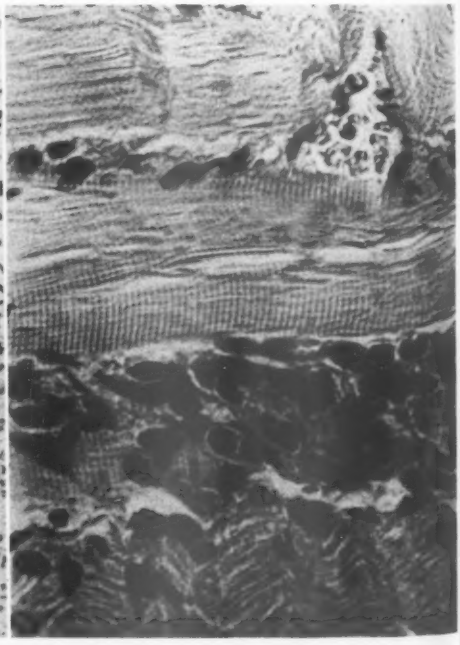
FIG. 7. Cross section of the human gastrocnemius muscle illustrated in Figure 4. Hematoxylin and eosin stain. × 516.

FIG. 8. Rat kidney illustrated in Figures 5 and 6. The organisms overlie an intact nucleus in a slightly different focal plane, but under the microscope were definitely within the cytoplasm. Steiner's stain. × 970.

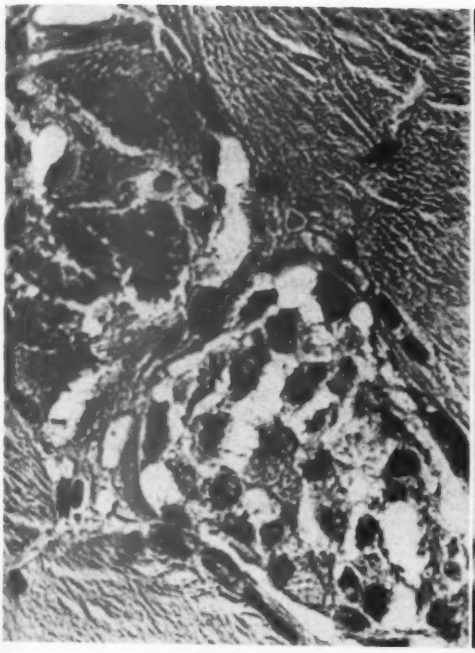
FIG. 9. Heart, lung, and kidney from a hamster which died of canicola fever. Of note is the absence of cortical markings on the sectioned kidney below the "butterfly" lungs. × 2½.



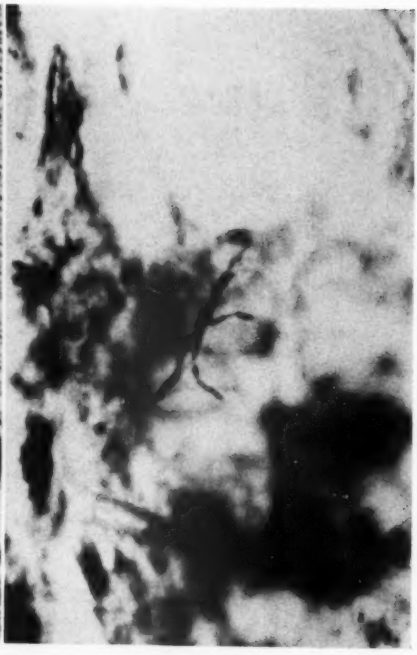
5



6



7



8

9

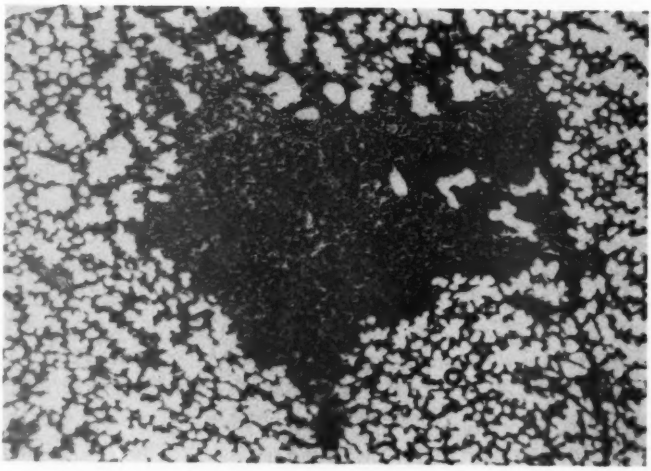
FIG. 10. Gastrocnemius muscle from a hamster which died of canicola fever. The hyalinized portions of three fibers lie below a zone of hemorrhage and the better preserved portions have retracted. Hematoxylin and eosin stain. × 516.

FIG. 11. Typical pulmonary hemorrhage in canicola fever from a guinea-pig which was normal to inspection while alive. Hematoxylin and eosin stain. × 60.

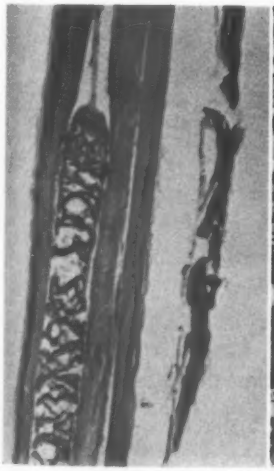
FIG. 12. Gastrocnemius muscle of a dog with proved leptospirosis of uremic type. Of note is the collapsed sarcolemma at the upper end of the degenerated fiber, the preservation of the adjacent fibers, and the marked interstitial edema. Hematoxylin and eosin stain. × 120.

FIG. 13. Gastrocnemius muscle of an icteric dog. One end of the damaged fiber and of the adjacent fibers is preserved. A normal segment of this same fiber was seen on the other end just beyond the edge of the field illustrated. Macrophages have begun débridement of the necrotic portion. Hematoxylin and eosin stain. × 120.

10



11



12

13

THE DESTRUCTIVE EFFECTS OF DL-ETHIONINE ON THE PANCREAS, STOMACH, AND SUBMAXILLARY GLANDS *

WILLIAM E. LORING, M.D., and LAWRENCE J. HARTLEY, M.D.
From the Department of Pathology, University of North Carolina School of Medicine, Chapel Hill, N.C.

Any compound that can cause selective cellular destruction within the body is an important and powerful research tool, and recent work has shown ethionine (alpha-amino-gamma-ethylmercaptobutyric acid) to be just such a material. Its specificity for the acinar cells of the pancreas with accompanying fatty metamorphosis of the liver has been described in detail.[1-10] Additional hepatic changes that include cholangitis, proliferation of the reticulo-endothelial system, and degeneration of hepatic cells have been described also by Koch-Weser and Popper[11] and Popper *et al.*[4] Alvizouri and Warren[10] found a florid proliferation of the bile ducts which in one case led to the formation of a cholangioma. De Almeida and Grossman[5] have reported the occurrence of submucosal hemorrhages in both the large and small bowel, and ulcers of the rectum. Fatty changes in the proximal convoluted tubules of the kidney have been described repeatedly.[3,4,8,10,12] Adrenal hemorrhage has been noted by Goldberg and Chaikoff.[3] Alvizouri and Warren also found rather marked degeneration of the seminiferous epithelium of the testis. An interesting observation by De Almeida and Grossman that the bleeding time in dogs, using the Lee-White method, was prolonged 30 to 40 minutes requires further investigation.

In all of the studies made so far, no changes involving the salivary glands, the mucosa of the stomach, or the duodenum have been mentioned. At least two reports, those by Goldberg and Chaikoff[3] and Alvizouri and Warren,[10] stated specifically that no changes were found in these organs.

In the experiments to be described, specific changes were found not only in the pancreas but also in the chief cells of the stomach and the duct cells and acini of the submaxillary glands. Those in the duodenal mucosa were less constant, but when they occurred they were quite marked.

MATERIAL AND METHODS

Twenty-six young albino rats of the Wistar strain, having an average weight of 134 gm., were maintained on a standard laboratory diet.

* Received for publication, October 8, 1954.

They were divided into six groups without regard to sex or weight. Group I (3 animals) acted as a control group. Group II (5 animals), group III (5 animals), and group IV (4 animals) were given 12.5, 25, and 50 mg. of DL-ethionine, respectively, by the intraperitoneal route every other day. Group V (4 animals) received 300 mg. of DL-ethionine intraperitoneally daily. In group VI (5 animals), 12.5 mg. of DL-ethionine were injected directly into the blood stream by intracardiac injection on the same schedule as for groups II to IV. The DL-ethionine was dissolved readily in normal saline solution when placed in a warm water bath to make a solution that contained 25 mg. per cc. All animals were transiently narcotized by ether just prior to handling to facilitate injection.

The animals were sacrificed and complete necropsies were performed after intervals varying from 8 hours to 41 days and after receiving 12.5 to 900 mg. of DL-ethionine (Table I). Representative sections of pancreas, liver, salivary gland, esophagus, stomach, small and large intestine, lung, bronchial glands, kidney, adrenal gland, spleen, testis, fallopian tube, ovary, uterus, lymph nodes, heart, and aorta were examined, using hematoxylin and eosin and Masson's trichrome stains. Zenker's solution and formalin and alcohol were used as fixatives. Sections of liver, kidney, and salivary gland were stained for fat using Sudan IV.

Results

Lethargy, weight loss, and loose stools in varying and increasing degrees were common ante-mortem findings. Although not detectable grossly, the most pronounced and interesting changes were found in the pancreas, stomach, and submaxillary glands.

Pancreas. Alteration of the acinar architecture of the pancreas was present in all animals, as is shown in Table I. It ranged from an individual cellular change consisting of loss of basal basophilia and swelling of the secretory granules to advanced dissolution of the acinar pattern with pyknosis, cell loss, and early replacement fibrosis. Those animals that were sacrificed early after the smaller doses of the drug (group II, nos. 1 and 2) showed the least changes while the more marked effects were found after larger doses over longer periods of time (group IV, nos. 2 and 3). The individual cells tended to become round or oval and to lie free. More frequently than not, two or three nuclei were present. As is shown in Figure 1, intracellular eosinophilic granules were still present even in those sections that showed marked alteration. In no case, however, was the pancreas completely destroyed. The infiltration by inflammatory cells was minimal or ab-

sent, although the interstitial tissues were somewhat edematous. The ducts and islets showed no significant change.

Stomach. The changes in the stomach mucosa involved the chief cells exclusively. These ranged from no detectable alteration in those

TABLE I

Summary of Total Dosage, Duration of Action, and Degree of Damage to Pancreas, Stomach, and Submaxillary Glands*

Rat no.	Total dosage (mg.)	Day of sacrifice	Pancreas	Stomach	Submaxillary gland	
					Ducts	Acini
Group II						
1	87.5	13	++	0	0	0
2	137.5	22	++	0	0	0
3	150	24	+++	++	0	+
4	175	28	+++	++	+	+
5	200	32	+++	++	+	++
Group III						
1	25	1	+++	+++	++	++
2	175	13	++	++	+	++
3	250	19	++	++	0	0
4	400	31	+++	++	++	+
5	425	41	++	++	++	+++
Group IV						
1	300	6	+++	+++	++++	+
2	350	7	++++	++	+	++
3	500	10	++++	++++	++++	+++
4	650	13	+++	+++	++++	+++
Group V						
1	300	1	++	+++	+++	+
2	300	1	+++	+++	++++	++
3	600	2	+++	+++	++++	++
4	900	3	+++	+++	++++	++
Group VI						
1	12.5	8 hours	++	0	++++	0
2	25	36 hours	+	0	++	0
3	62.5	10	++	0	+	0
4	112.5	18	++	++	++++	+
5	200	32	++	0	+++	+

* Degree of damage to cells and of alteration of the pattern of ducts or acini is indicated by 0 to ++++.

rats that were on the lower dose schedule and sacrificed early (group II, nos. 1 and 2) to almost total loss in those animals receiving higher doses over longer periods (groups IV and V, Table I). The cellular changes appeared to follow a general course that started with edema and increased granularity of the cytoplasm, and progressed through vacuolization and disruption of glandular pattern to complete dissolution (Figs. 2 and 3). The nuclei of those cells showing early changes were not remarkable, but as the destruction progressed they became pyknotic and karyolytic with final dispersion of their fragments. These

changes were most marked in the body chief cells, while the neck chief cells showed much less reaction. The parietal cells, although occupying adjacent positions, remained histologically unchanged (Fig. 3). An inflammatory reaction was absent. In those animals in which the chief cells had completely disappeared, the remaining fine reticular supporting structure showed no sign of proliferation.

Salivary Glands. The studies of the salivary glands were concentrated on the submaxillary and sublingual glands, although representative sections of the parotid glands were examined. Reactive changes were most marked in the submaxillary glands and were found in all but those animals receiving low doses of ethionine with early sacrifice (group II, nos. 1 and 2). These alterations involved principally the smaller ducts and to a lesser degree the acini. As would be expected, they were more striking in those animals that had received the larger doses (groups IV and V, Table I).

The intralobular and intercalary segments of the ducts were most severely damaged (Figs. 4 and 5). The cytoplasmic changes in the duct cells consisted of hydrops, increased granularity with a reduction in affinity for eosin, vacuolization, loss of cellular integrity, amorphous free-floating masses, and complete dissolution (Fig. 5). The nuclear changes included pyknosis, leading to karyorrhexis and in some instances karyolysis. The basement membranes showed no detectable changes.

The cells of the interlobular segments of the ducts contained examples of mild hydrops and some increased granularity in the high-dose animals, but nuclear changes were minimal.

The alterations of the acinar cells also were much less marked. They consisted principally of cloudy swelling, increased granularity of the cytoplasm, and disruption of the normal acinar pattern. The nuclear changes were not remarkable except for occasional reduplication and alteration of their normal position in the cell.

The reaction in the parotid glands was inconstant and much less marked. The sublingual glands were free of any detectable histologic variation.

Duodenum. The duodenal changes were not consistent. Varying degrees of damage were found in cells both of the mucosa and of Brunner's glands. An example of marked change is shown in Figure 6. Cellular changes were marked in all animals of group IV and in rat 2 of group V. The pattern of destruction resembled that described for gastric cells.

It was of some interest that only 4 animals (group II, no. 1; group III, nos. 3 and 5; group VI, no. 3) of the entire series showed lipidosis of the liver. The fat droplets were minimal and intracellular.

Fat was present in the cells of the proximal convoluted tubules of the kidney in the animals receiving higher doses, but this also was not marked.

Multiple sections taken from the testes and the remaining organs were not remarkable.

Discussion

There is now good experimental evidence to show that DL-ethionine exerts its toxic effects by inhibiting the incorporation of methionine and glycine into the proteins of some tissues[13] and that the substituted molecule will not only not support growth[14] but will cause specific tissue destruction.[1-10]

When one considers the close resemblance in structure and function of the pancreatic acini, the chief cells of the stomach, and the serozymogenic cells of the salivary glands, these findings are not surprising. It would be logical to assume that a substance, such as ethionine, that has a proved specific vitiating effect on the pancreas would also injure the other two.

The pathologic physiology of the pancreas damaged by ethionine has been studied in some detail.[13-17] Further similar studies are now needed for the stomach and salivary glands.

Pancreas. Although the total dosage of ethionine ranged as high as 900 mg., none of the pancreatic sections examined showed the degree of destruction previously reported on equal or lesser total doses. This probably can be attributed to the short recovery periods allowed by the injection regimen of every other day.

It is of interest that the most striking nuclear changes—those of prominent nucleoli, chromatin clumping and "ringing," and multiple nucleation of the acinar cells—gave the impression of increased cellular activity rather than destruction. As is seen in Table I, those animals that showed advanced cellular destruction were in the minority.

Stomach. In the stomach the damage to the chief cells was much more complete than in the analogous cells of the pancreas. It is worthwhile to emphasize that the chief cells were specifically destroyed while the adjacent parietal and mucous neck cells were histologically unchanged. Wachstein and Meisel[9] have suggested that after destruction, the pancreatic acini regenerate from the dedifferentiated fibroblast-like remaining acinar cells. This observation could not be

transferred to the stomach, for in our sections there was no evidence of attempted regeneration from either the remaining chief cells or stromal cells.

Salivary Glands. Stormont,[18] writing in Cowdry and citing Pischinger,[19] stated that the intercalary and intralobular ducts may be considered secretory in nature. The findings in this series of experiments would tend to confirm this observation. It is noteworthy that the degree of destruction of the acinar cells was much less marked than that of the ducts. The explanation for this is not readily forthcoming and requires further investigation.

Liver. Although the total dosage of ethionine was comparable to that given in previously reported experiments, lipidosis of the liver was minimal in our series. In view of the relatively unaltered pancreas, this is not surprising. It would seem that liver damage is secondary to pancreatic destruction rather than a direct toxic effect.

Duodenum. The effects on the duodenum are difficult to evaluate. Their limitation to groups IV and V imply that the larger doses of ethionine are important in their production. The period of time over which the drug is given is probably of lesser significance.

A concentrated search was made for the presence of generalized thrombosis and increased tendency to hemorrhage in the presence of pancreatic damage, and none was found. Although bleeding and clotting times were not specifically obtained, there was a singular lack of bleeding tendency following the multiple direct intracardiac injections.

The question as to whether ethionine would be utilized more rapidly and effectively if it was injected directly into the blood stream was considered. As is shown by the results in group VI in Table I, no clear-cut differences were found. Although the degree of pancreatic damage was slightly less marked, in 3 animals the destructive effect on the submaxillary gland ducts equalled that found in animals that were the recipients of much higher doses. Again, the explanation awaits further investigation.

The problem of mucoviscidosis, such as occurs in pancreatic inadequacy of the celiac syndrome, was also considered. In only one animal (group IV, no. 3) was any alteration of the bronchial glands found. Sections of these showed them to be widely dilated and filled with a homogeneous eosinophilic material (Fig. 7).

Summary

Twenty-six rats of the Wistar strain were divided into six groups without regard to sex or weight. One group acted as a control. The

remaining five received varying doses of DL-ethionine while being maintained on a standard laboratory diet. In one of the five groups, it was given by the intracardiac route. The remaining four received the drug intraperitoneally. With the exception of one high-dose group, an injection regimen of every second day was employed. All animals were sacrificed after periods varying from 6 hours to 41 days and after total dosage of 125 to 900 mg. Complete tissue examinations revealed a specific destructive effect on the acinar cells of the pancreas, the chief cells of the stomach, and both the ductal and acinar cells of the submaxillary glands. Previously described changes in the liver, kidneys, testes, and adrenal glands were not found.

REFERENCES

1. Farber, E., and Popper, H. Production of acute pancreatitis with ethionine and its prevention by methionine. *Proc. Soc. Exper. Biol. & Med.*, 1950, **74**, 838–840.
2. Goldberg, R. C.; Chaikoff, I. L., and Dodge, A. H. Destruction of pancreatic acinar tissue by DL-ethionine. *Proc. Soc. Exper. Biol. & Med.*, 1950, **74**, 869–872.
3. Goldberg, R. C., and Chaikoff, I. L. Selective pancreatic acinar destruction by DL-ethionine. *A. M. A. Arch. Path.*, 1951, **52**, 230–238.
4. Popper, H.; de la Huerga, J., and Koch-Weser, D. Hepatic and pancreatic changes produced in rats by ethionine and their relation to human lesions. (Abstract.) *Am. J. Path.*, 1952, **28**, 518–519.
5. De Almeida, A. L., and Grossman, M. I. Experimental production of pancreatitis with ethionine. *Gastroenterology*, 1952, **20**, 554–577.
6. Fitzgerald, P. J., and Alvizouri, M. Rapid restitution of the rat pancreas following acinar cell necrosis subsequent to ethionine. *Nature, London*, 1952, 170, 929–930.
7. Popper, H., and de la Huerga, J. Hepatic and pancreatic lesions due to conditional amino-acid deficiency. *Proc. Inst. Med. Chicago*, 1952–53, **19**, 152–153.
8. Wachstein, M., and Meisel, E. Equal effectiveness of L and D-ethionine in producing tissue damage in rats and mice. *Proc. Soc. Exper. Biol. & Med.*, 1953, **82**, 70–72.
9. Wachstein, M., and Meisel, E. Cellular changes accompanying the degenerative and regenerative phase of ethionine-induced pancreatic damage in the rat. *Lab. Investigation*, 1953, **2**, 253–260.
10. Alvizouri, M., and Warren, S. Effects of DL-ethionine on the pancreas and other organs. *A. M. A. Arch. Path.*, 1954, **57**, 130–137.
11. Koch-Weser, D., and Popper, H. Hepatic fibrosis produced by chronic ethionine feeding. *Proc. Soc. Exper. Biol. & Med.*, 1952, 79, 34–37.
12. Wachstein, M., and Meisel, E. Nephrotoxic action of DL-ethionine. *Proc. Soc. Exper. Biol. & Med.*, 1951, **77**, 648–651.
13. Simpson, M. V.; Farber, E., and Tarver, H. Studies on ethionine. I. Inhibition of protein synthesis in intact animals. *J. Biol. Chem.*, 1950, **182**, 81–89.

14. Kalser, M. H., and Grossman, M. I. Pancreatic secretion in dogs with ethionine-induced pancreatitis. *Gastroenterology*, 1954, **26**, 189–197.

15. Lin, T. M., and Grossman, M. I. Reversal by DL-methionine of acute effect of DL-ethionine on pancreatic enzyme output in dogs. *Am. J. Physiol.*, 1954, **176**, 377–380.

16. Feinberg, H.; Rubin, L.; Hill, R.; Entenman, C., and Chaikoff, I. L. Reduction of serum lipides and lipoproteins by ethionine feeding in the dog. *Science*, 1954, **120**, 317–318.

17. Farber, E.; Simpson, M. V., and Tarver, H. Studies on ethionine. II. The interference with lipide metabolism. *J. Biol. Chem.*, 1950, **182**, 91–99.

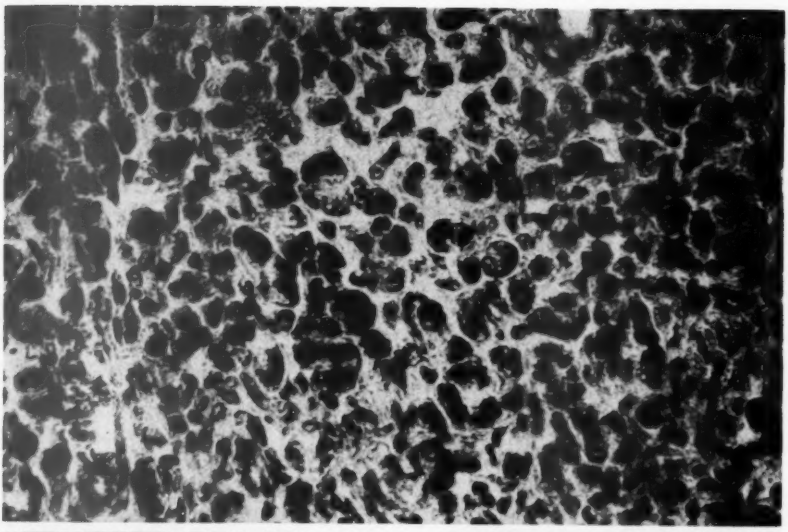
18. Stormont, D. L. The Salivary Glands. In: Cowdry, E. V. (ed.) Special Cytology. Paul B. Hoeber, Inc., New York, 1932, ed. 2, p. 174.

19. Pischinger, A. Beiträge zur Kenntnis der Speicheldrüsen, besonders der Glandula sublingualis und submaxillaris des Menschen. *Ztschr. f. mikr.-anat. Forsch.*, 1924, Abt. 2, 1, 437–489.

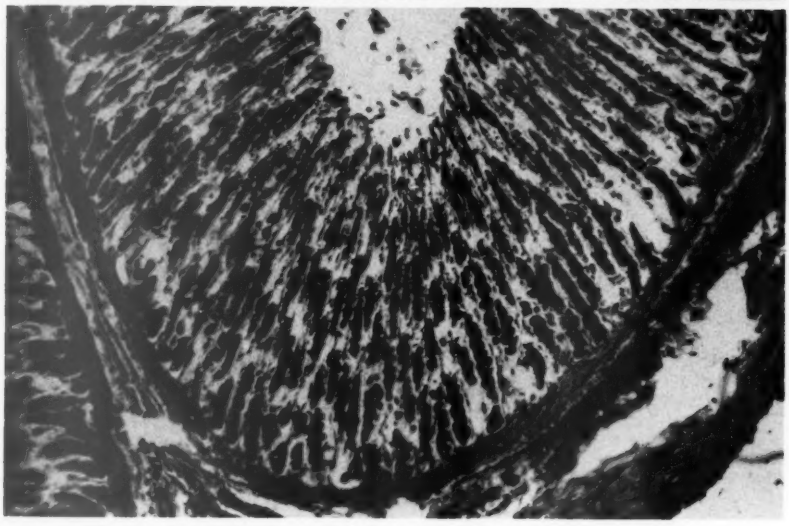
LEGENDS FOR FIGURES

FIG. 1. Pancreas. Loss of normal acinar pattern with prominent eosinophilic intracellular granules in the round and oval acinar cells. Hematoxylin and eosin stain. × 450.

FIG. 2. Stomach. Low-power photomicrograph showing disturbance of normal glandular pattern and cell loss in the deeper mucosal levels. Hematoxylin and eosin stain. × 100.



1



2

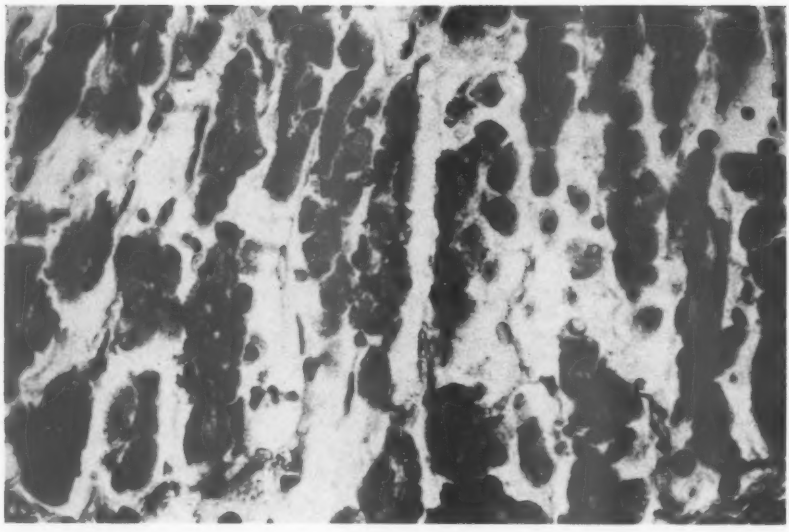
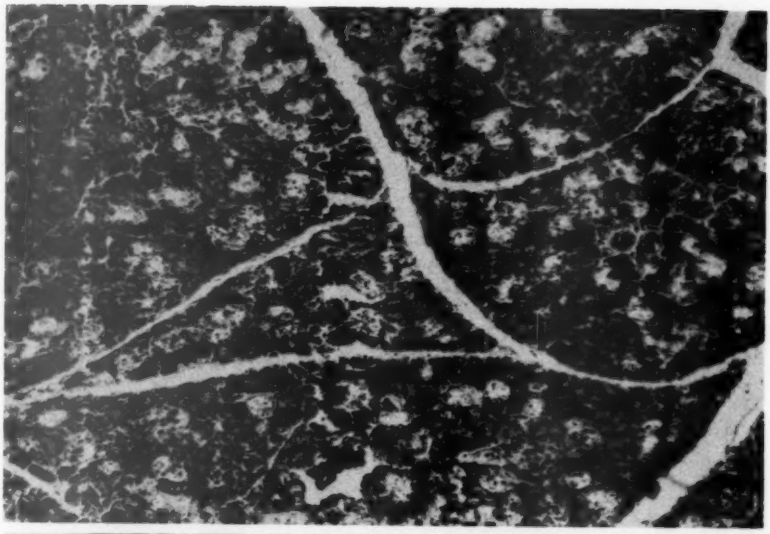


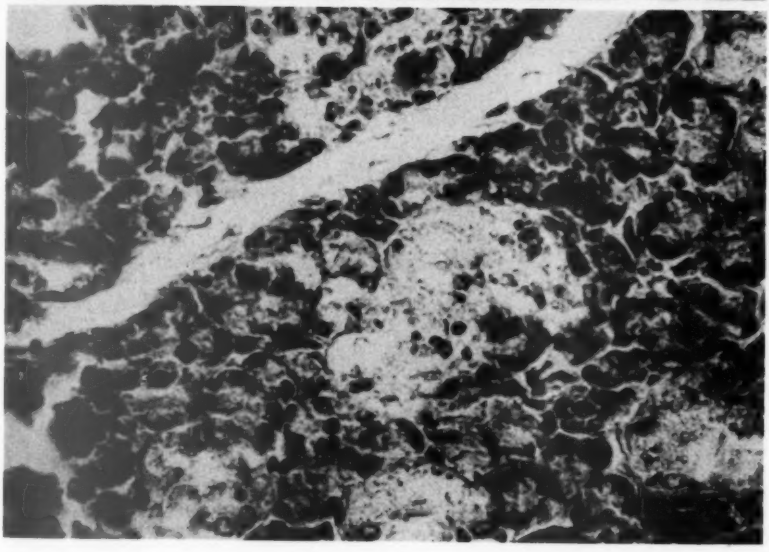
Fig. 3. Stomach. High-power view of Figure 2 showing selective damage and loss of chief cells. Parietal cells appear unaffected. Hematoxylin and eosin stain. × 450.

Fig. 4. Submaxillary gland. Low-power photomicrograph showing widespread involvement of ducts. Hematoxylin and eosin stain. × 100.

Fig. 5. Submaxillary glands. High-power view of Figure 4. Marked destruction of the epithelium lining the ducts with a lesser effect on the adjacent acinar cells. Hematoxylin and eosin stain. × 450.



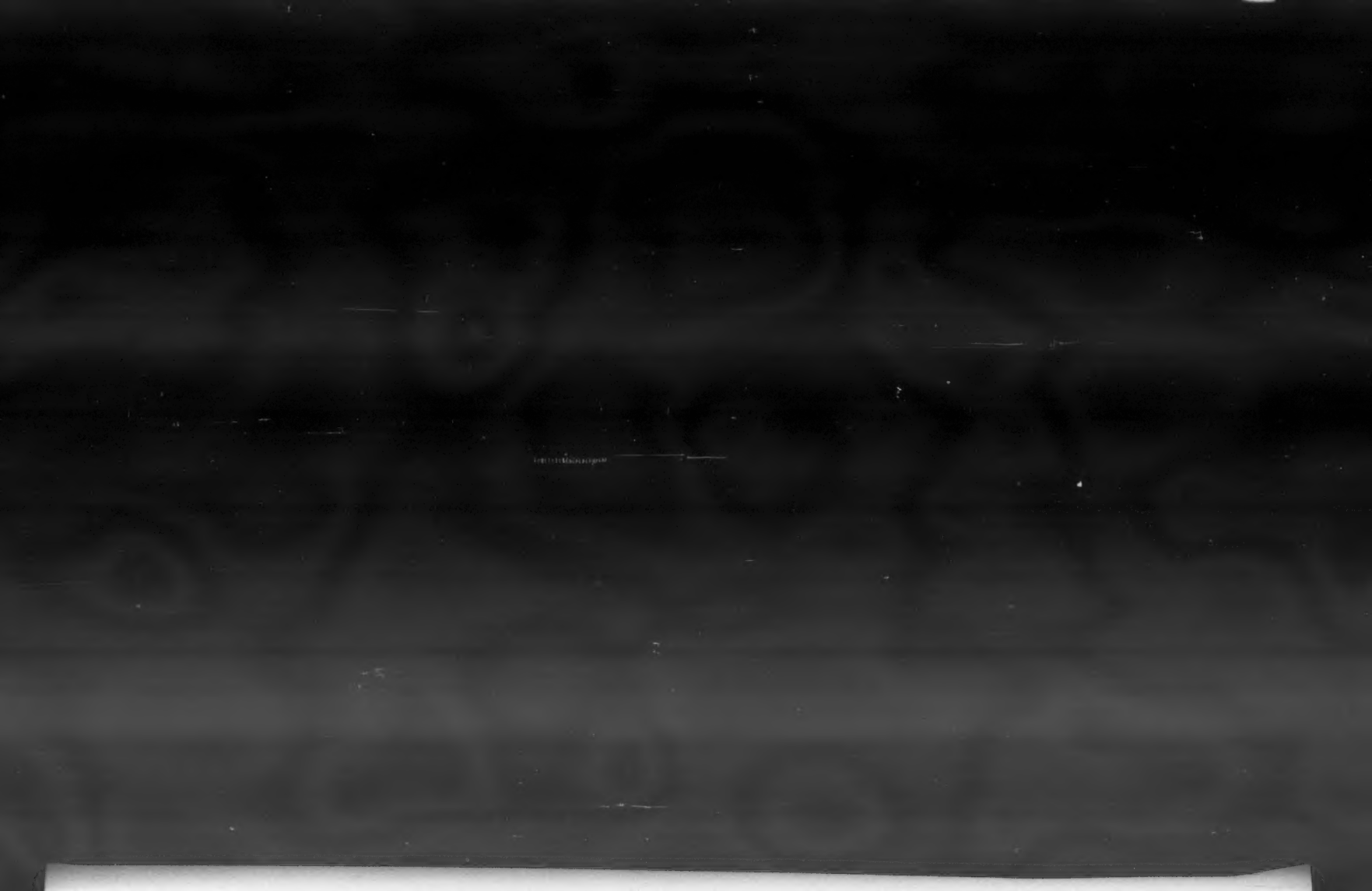
4

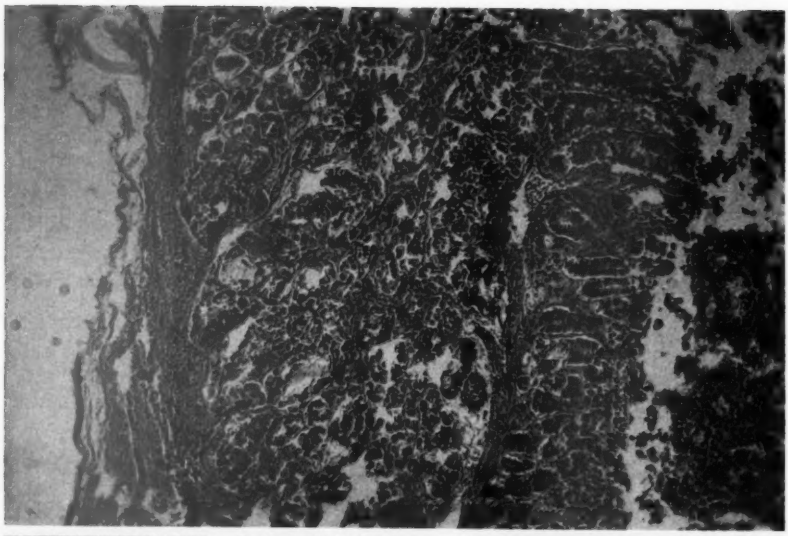


5

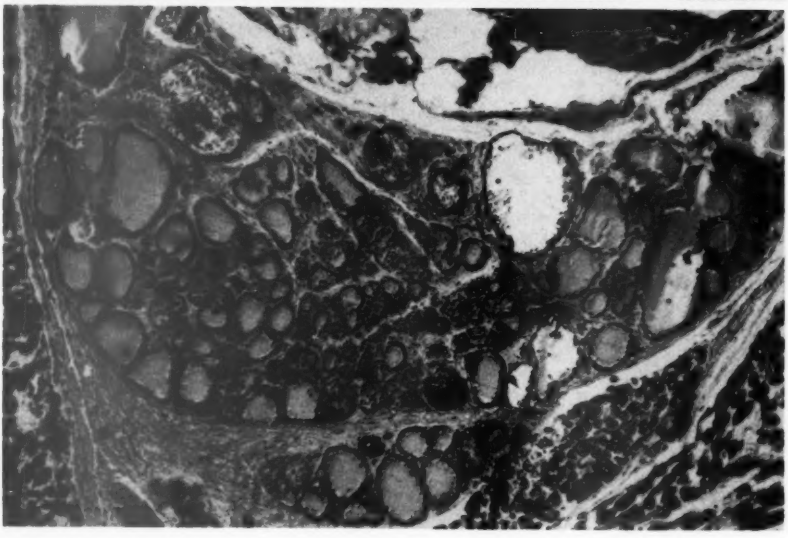
FIG. 6. Duodenum. The glandular pattern of Brunner's glands at the origin of the duodenum is disrupted. Changes in the overlying duodenal mucosa are also marked. Hematoxylin and eosin stain. × 100.

FIG. 7. Bronchial glands. Glands are widely dilated and their lumina are filled with a homogeneous eosinophilic material. Hematoxylin and eosin stain. × 100.





6



7

CORTISONE IN EXPERIMENTAL HISTOPLASMOSIS*

RALPH A. VOGEL, Ph.D.; MAX MICHAEL, JR., M.D.,† and ALICE TIMPE, M.A.‡
From the Laboratory (Dr. Vogel and Miss Timpe) and Medical (Dr. Michael) Services, Veterans Administration Hospital, and the Department of Medicine (Dr. Michael), Emory University School of Medicine, Atlanta, Ga.

The deleterious effects of cortisone and ACTH on the course of both clinical and experimental infections is well documented.[1] Experimental *Blastomyces dermatitidis* and *Candida albicans* infections are enhanced in mice by cortisone treatment; and those of *Coccidioides immitis* are enhanced in the rat,[1] but minimally affected in the mouse.[2] This report deals with the effect of cortisone treatment on the serologic response and pathologic picture of guinea-pigs infected with the yeast phase of *Histoplasma capsulatum.*

MATERIALS AND METHODS

Pigmented guinea-pigs, weighing approximately 300 gm., and of either sex, were used in these studies. Ten animals comprised each of the following groups:

Group A: Infected with *H. capsulatum,* and treatment with cortisone commenced at the same time. Treatment discontinued 2 weeks before necropsy.

Group B: Infected with *H. capsulatum,* and treatment with cortisone commenced 2 weeks later. Treatment continued until necropsy.

Group C: Infected with *H. capsulatum* and given no treatment.

Group D: Normal animals treated with cortisone.

Group E: Normal animals.

Infecting Dose. The yeast phase of a strain of *H. capsulatum,* recently isolated from a patient having a complement fixation titer to *H. capsulatum* of 1:32,768, was grown on brain-heart-infusion blood agar. A dose of 0.5 ml. of a 1:100 concentration by volume of yeast cells was given intraperitoneally to all but those of groups D and E. This dose was shown by Howell[3] to result in a chronic infection in which mortality was negligible, but positive spleen cultures could be obtained months after infection. Cortisone was administered daily for 45 days in a dose of 5 mg. by intraperitoneal injection.

Serologic and Skin Tests. Blood was drawn by cardiac puncture from all animals prior to infection and during the second, fifth, and

* Received for publication, September 13, 1954.

† Now at State University of New York College of Medicine, New York, N.Y., and Maimonides Hospital, Brooklyn, N.Y.

‡ Now at Alaska Native Service Hospital, Anchorage, Alaska.

seventh weeks following infection and on the ninth week just before necropsy. The serum was tested for complement fixing antibodies to Histoplasmin* according to the methods designed by Kent *et al.*[4] Complement was titrated to the end point of 50 per cent hemolysis, where an E.P. 50 unit represents the amount of complement giving 50 per cent lysis of a given amount of sensitized sheep red cells. The amount of hemolysis was measured on a Coleman Junior Spectrophotometer at 550 mμ.

Veronal buffered saline solution was used as diluent for the antigen and antiserum, and in the preparation of complement. The stock solution of buffer contained 85.0 gm. of NaCl, 5.75 gm. of 5,5 diethyl barbituric acid, and 3.75 gm. of Na-5,5 diethyl barbiturate. The acid, dissolved in 500 ml. of hot water, was added to the solution of the other constituents and the final volume adjusted to 2000 ml. with water. The working solution was prepared by adding one part of stock to four parts of water (pH 7.3 to 7.4).

Guinea-pig serum was inactivated at 56° C. for 30 minutes and diluted 1:3 with veronal buffer. Three-tenths ml. of this dilution, containing 0.1 ml. of serum, was designated as the 1:1 dilution in the test. Serial dilutions were made from the 1:3 dilution and used in 0.3 ml. amounts.

Complement containing three 50 per cent hemolytic units in 0.3 ml. and 0.3 ml. of the optimal antigen dilution (1:50) of Histoplasmin were employed. After an 18-hour incubation period at 4° C., 0.6 ml. of optimally sensitized sheep red cells were added to the test. The highest dilution of serum showing 50 per cent hemolysis or less was considered the titer.

Histoplasmin was diluted 1:10 for skin testing purposes.

Termination of the Experiment. All animals were killed by chloroform inhalation at the end of 60 days of observation and necropsied immediately. Macroscopic observations were made and sections from liver, spleen, adrenal gland, and lungs were removed for histologic examination. Two tubes of Sabouraud's agar were inoculated from the spleen: one, by an impression smear of the cut surface, and the other with a piece of the organ.

Results

No adverse effects, such as weight loss, increased mortality, or morbidity, were noticed in any group during the observation period. The death of two animals from the cortisone treated group 4 weeks

* Furnished by Parke, Davis & Co., Detroit, Mich.

TABLE I

Observations on Guinea-Pigs Infected with Histoplasma capsulatum and Treated with Cortisone for 45 Days

Group	Skin test* (mm.) I	E	Serologic titer to histoplasmin (weeks)† 0	2	5	7	9	Culture (spleen)‡	Tissue involvement (organisms) Spleen	Adrenal gland	Lung	Liver
A												
I-1§			0	0	64			++++	Many	Many	Many	Many
I-2	17	13	0	0	16	64	32	++++	Few	—	—	—
I-3	10	9	0	0	0	0	0	+	—	—	—	—
I-4	0	0	0	0	128	128	64	++++	—	—	—	—
I-5	5	5	0	0	0	0	0	++++	Many	Few	Few	—
IV-1	7	6	0	0	0	0	0	++++	Many	Many	Many	—
IV-2	10	8	0	0	64	64	0	++	Few	—	—	—
IV-3	0	8	0	0	16	16	0	++++	Few	—	—	—
IV-4§			0	0	16			++++	—	—	—	—
IV-5	8	7	0	0	64	64	16	++++	Few	—	—	—
B												
VIII-1	14	14	0	0	0	64	32	+	—	—	—	—
VIII-2	14	14	0	0	0	0	0	++++	Many	Many	Many	Many
VIII-3	0	5	0	0	128		32	++++	Few	Few	—	Few
VIII-4	17	7	0	0	32	32	64	++++	Few	Few	—	Few
VIII-5	9	8	0	0	0	0	0	+	—	—	—	—
IX-1	7	6	0	0	64	64	32	++++	Many	Many	Many	Many
IX-2	7	4	0	0	16	64	64	++++	Many	Many	Many	Many
IX-3	15	13	0	0	8	32	16	++++	Few	—	—	—
IX-4	5	5	0	0	32	0	0	+++	Few	—	—	—
IX-5	8	6	0	0	32	32	16	+	—	—	—	—
C												
V-1	13	13	0	0	32	32	0	+	—	—	—	—
V-2	16	12	0	0	32	64	16	+	—	—	—	—
V-3	18	15	0	0	8	32	16	+	—	—	—	—
V-4	15	12	0	0	32	32	32	+++	—	—	—	—
V-5	12	11	0	0	0	0	0	+	—	—	—	—
VI-1	0	10	0	0	8	8	4	+	—	—	—	—
VI-2	15	14	0	0		32	32	+++	Few	—	Few	—
VI-3	0	0	0	0	0	128	256	+++	Many	Many	Many	—
VI-4	20	20	0	0	32		16	+	—	—	—	—
VI-5	14	12	0	0	16	64	16	+	—	—	—	—

Group A: Treated with cortisone immediately.
Group B: Cortisone treatment delayed.
Group C: No cortisone.
* I = induration, E = erythema.
† Reciprocal of titer.
‡ 4 plus = heavy growth.
§ Died.

after infection is not considered significant despite evidence of disseminated infection, since they represented a small percentage of the total and their deaths did not exceed the normal expected death rate. All animals were skin-tested 5 weeks following infection. The untreated controls, as well as the cortisone controls, were uniformly negative. Strong positives were noted in the group infected with *H. capsulatum* (group C); these reactions appeared somewhat more intense than in those infected and treated with cortisone (groups A and B) (Table I). Since the results in groups D and E were uniformly negative for each category of the table, the findings have not been tabulated.

The results of the complement fixation tests failed to show any effects of cortisone treatment on the time of appearance or in the height of titer in Histoplasma infected guinea-pigs. The titers of animals in group A, in which cortisone treatment was discontinued 2 weeks before necropsy, fell off significantly at the last bleeding (Table I). While a decrease in titer was noted in all other groups during the ninth week, it was not so pronounced. The significance of this finding remains obscure.

Cultures from the spleen were observed daily for appearance and extent of growth. At the end of 1 week of incubation at room temperature, 8 of 10 cultures from group A and 7 of 10 from group B showed heavy early growth, while only 2 of 10 from group C showed comparable growth. At the end of 3 weeks (Figs. 1, 2, and 3), it was readily apparent that cultures made from the impression smears from cortisone treated animals were more heavily seeded with organisms.

Histopathologic Examination. There was no difference in the gross appearance of organs from cortisone treated and untreated animals. Hematoxylin and eosin sections, however, revealed a greater dissemination of the organisms in the cortisone treated groups, which was in agreement with the results obtained by culturing the tissues.

Discussion

Cortisone therapy in the management of clinical histoplasmosis has recently been discussed.[5] Since reports are conflicting and too few in number to assay its rôle in this disease, a more thorough laboratory evaluation of the situation was considered necessary. Furthermore, because of the many similarities between some cases of sarcoidosis and histoplasmosis,[6] many patients with the latter disease may receive cortisone which would be indicated were their disease sarcoidosis. In-

vestigation of the serologic response of animals under cortisone treatment was also suggested when it was noted recently that a patient with disseminated histoplasmosis had an exceptionally high titer of 1:32,768 in a complement fixation test after cortisone therapy.

The guinea-pig was selected because of its general resistance to Histoplasma infection and tendency to harbor the organisms for long periods of time. Under these conditions, it was believed that exacerbation under cortisone treatment might be demonstrated by increased mortality and morbidity. That the animals did not show clear-cut signs of widespread dissemination or of unusual serologic activity should not be taken as unequivocal proof that this cannot happen since the pathologic and serologic responses to cortisone treatment have been shown to depend largely on the species of animal studied.[1,7] Some dissemination of the organism undoubtedly occurred, as shown by cultural studies and the histopathologic examination; but either the natural resistance of the guinea-pig to this disease or some protective tissue activity due to cortisone treatment blocked a lethal spread of the organism.

In recent studies, the similarity and coexistence of sarcoidosis and histoplasmosis have been discussed.[6,8] In a series of 29 cases of sarcoidosis studied at the Memphis Veterans Administration Hospital, no evidence of Histoplasma could be found.[9] These patients, however, did not receive cortisone therapy. Whether or not they would have developed histoplasmosis superimposed on sarcoidosis had they received cortisone is an interesting speculation, since Prior *et al.*[10] have shown that the unimmunized dog pretreated with cortisone succumbs to an intratracheally introduced mycelial inoculum. In a region where Histoplasma is endemic, the prolonged use of cortisone in patients with sarcoidosis who do not have immunity to histoplasmosis may result in an increase of coexisting sarcoidosis and histoplasmosis.

Summary

Guinea-pigs infected with Histoplasma were given immediate and delayed cortisone treatment. No significant difference in mortality or morbidity was observed in either group of animals compared with untreated controls over a period of 60 days. Evidence of wider dissemination of the organism in cortisone treated animals was noted in impression smear cultures of the spleen and microscopic examination of hematoxylin and eosin smears. The serologic response of the treated and untreated groups was essentially the same.

REFERENCES

1. Kass, E. H., and Finland, M. Adrenocortical hormones in infection and immunity. *Ann. Rev. Microbiol.*, 1953, **7**, 361–388.
2. Newcomer, V. D.; Wright, E. T.; Tarbet, J. E.; Winer, L. H., and Sternberg, T. H. The effects of cortisone on experimental coccidioidomycosis. *J. Invest. Dermat.*, 1953, **20**, 315–327.
3. Howell, A., Jr. Isolation of pathogenic fungi from experimentally inoculated guinea pigs. *Pub. Health Rep.*, 1948, **63**, 602–616.
4. Kent, J. F.; Bukantz, S. C., and Rein, C. R. Studies in complement fixation. I. Spectrophotometric titration of complement; construction of graphs for direct determination of the 50 per cent hemolytic unit. *J. Immunol.*, 1946, **53**, 37–50.
5. Michael, M., Jr., and Vogel, R. A. Histoplasmosis: report of a case, with observations on management. *New England J. Med.*, 1954, **251**, 884–887.
6. Israel, H. L.; DeLamater, E.; Sones, M.; Willis, W. D., and Mirmelstein, A. Chronic disseminated histoplasmosis. Investigation of its relationship to sarcoidosis. *Am. J. Med.*, 1952, **12**, 252–260.
7. Mirick, G. S. The effects of ACTH and cortisone on antibodies in human beings. *Bull. Johns Hopkins Hosp.*, 1951, **88**, 332–351.
8. Pinkerton, H., and Iverson, L. Histoplasmosis. Three fatal cases of disseminated sarcoid-like lesions. *A. M. A. Arch. Int. Med.*, 1952, **90**, 456–467.
9. Gendel, B. R.; Young, J. M., and Greiner, D. J. Sarcoidosis. Review with 24 additional cases. *Am. J. Med.*, 1952, **12**, 205–218.
10. Farrell, R. L.; Cole, C. R.; Prior, J. A., and Saslaw, S. Experimental histoplasmosis. I. Methods for production of histoplasmosis in dogs. *Proc. Soc. Exper. Biol. & Med.*, 1953, **84**, 51–54.

LEGENDS FOR FIGURES

FIG. 1. Cultures from guinea-pigs infected with *Histoplasma capsulatum* and started on cortisone at the time of infection. Not shown are two cultures with heavy growth from guinea-pigs which died early in the experiment.

FIG. 2. Cultures from guinea-pigs infected with *H. capsulatum* and started on cortisone 2 weeks later.

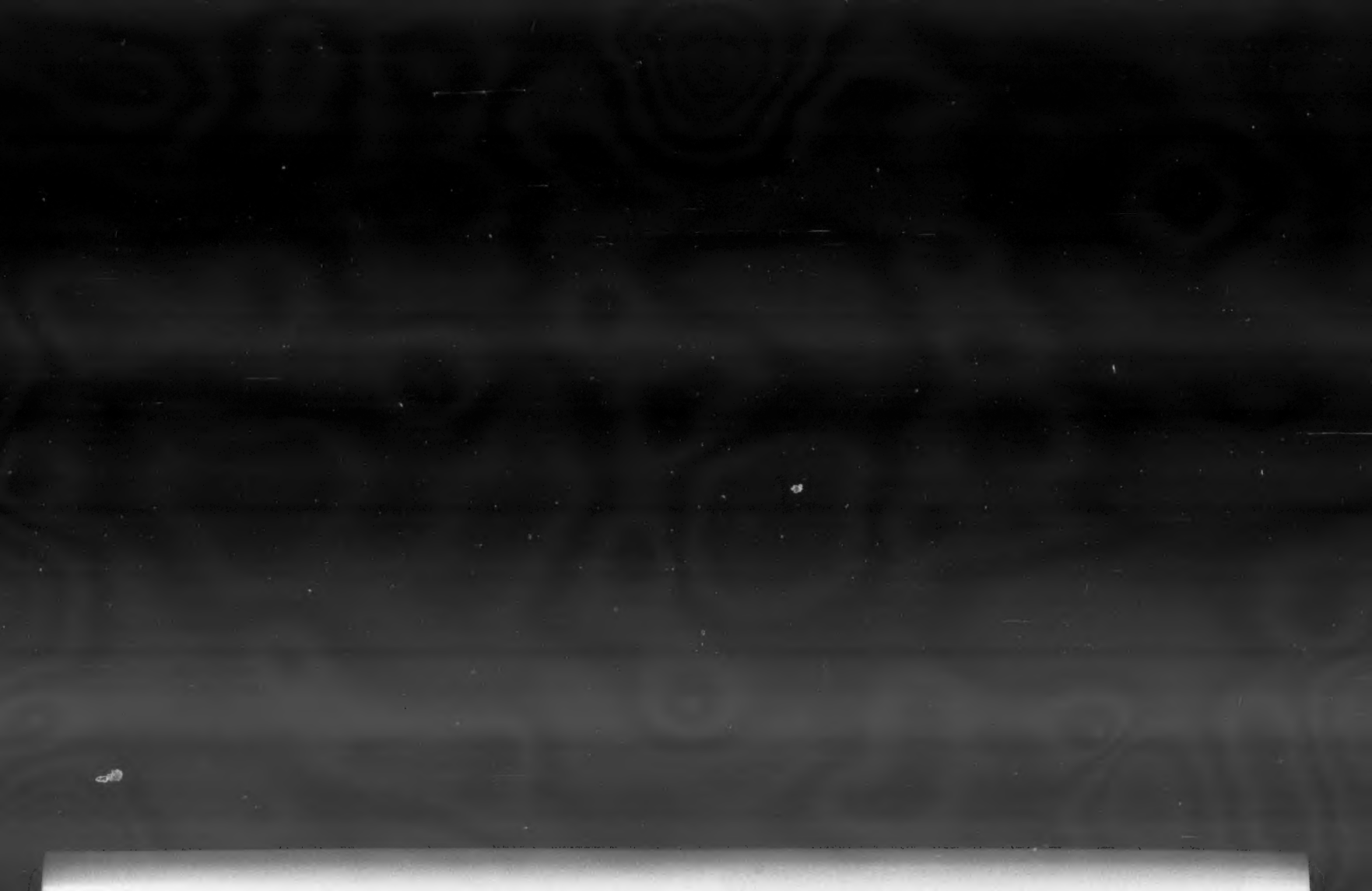

12
13
14
15
41
42
43
45
GROUPS I & IV
INFECTED WITH HISTOPLASMA CAPSULATUM

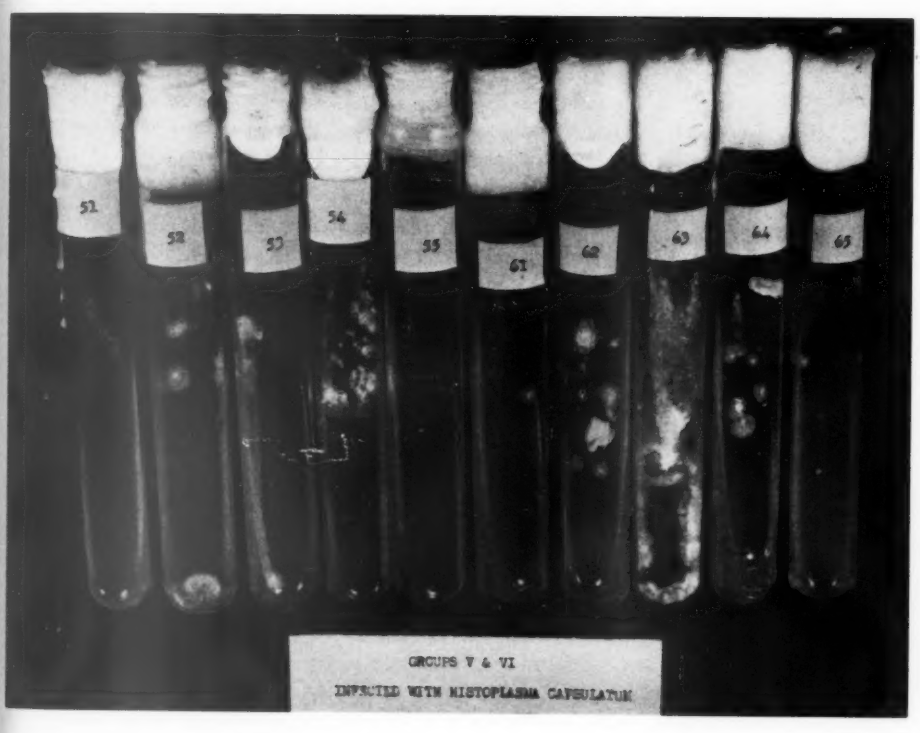
1


91
92
93
94
95
GROUPS VIII & IX

2

FIG. 3. Cultures from guinea-pigs infected with *H. capsulatum* and not treated with cortisone.




52
58
53
54
55
61
62
63
64
65
GROUPS V & VI
INFECTED WITH HISTOPLASMA CAPSULATUM

3

HISTOCHEMICAL DEMONSTRATION OF COPPER IN A CASE OF HEPATOLENTICULAR DEGENERATION *

CHARLES L. GREEN, M.D.

From the Veterans Administration Hospital, McKinney, Texas, and Southwestern Medical School of the University of Texas, Dallas, Texas

Interest in the rôle of copper in hepatolenticular degeneration has existed since Rumpel[1] (1913) demonstrated an increased amount of copper in the liver from a case of Wilson's disease. Subsequent studies have shown that there is also increased retention in the brain. This paper is a report of the demonstration of copper in tissues from a case of hepatolenticular degeneration by histochemical methods.

Westphal,[2] in 1883, described a patient with a disease of the central nervous system characterized by choreo-athetoid movements and rigidity. Several years later, Strümpell[3] reported upon a group of children with a similar clinical picture, and referred to the condition as Westphal's pseudosclerosis. In 1912, Wilson[4] published a paper entitled "Progressive Lenticular Degeneration." His patients had a progressive familial disease. The main pathologic findings were cirrhosis of the liver and bilateral degeneration of the basal ganglia. Spielmeyer,[5] in 1920, suggested that Westphal, Strümpell, and Wilson had reported variations of the same disease. In 1921, Hall[6] grouped Westphal-Strümpell's pseudosclerosis and Wilson's disease as one entity, which he called hepatolenticular degeneration.

While investigating heavy metal intoxication in relation to the disease, Rumpel[1] (1913) reported an increased concentration of copper in the liver of a patient dying of Wilson's disease. Haurowitz[7] (1930) also reported an increased amount of copper in the liver and brain of his patient. Glazebrook[8] (1945) and Cumings[9] (1948) studied the copper content of liver and brain and found an abnormally high deposition in association with hepatolenticular degeneration, as compared with normal tissues. Cumings reported retention of copper in the cerebral gray and white matter, caudate nucleus, globus pallidus, and putamen. The highest copper concentrations were found in the liver and putamen.

REPORT OF CASE

The patient was a white male, 24 years old, who had been in good health until 2 years prior to his death. Symptoms of progressive central nervous system disease developed, characterized by rigidity, chorea, and athetoid movements.

Physical examination revealed facial grimace, mask-like facies at times, severe muscle spasticity, slow clonic muscle tremors, and incoordination of arms and legs. The patient was mentally clear, but had difficulty in speaking and swallowing. Pupil

* Received for publication, December 7, 1954.

and tendon reflexes were normal. A greenish pigmentation was present bilaterally along the peripheral corneal margin (Kayser-Fleischer ring). Large, reddish brown freckles covered the entire body; no other pigmentation was present. The heart and lungs were not remarkable, the liver and spleen were not palpable, and there was no unusual lymphadenopathy. Roentgenograms of the skull were normal. An electroencephalogram revealed symmetrical, 6.5 per second, frontoparietal bursts and runs. This was interpreted as consistent with subcortical lesions of frontoparietal ophthalmocortical circuits.

The total serum proteins were 5.3 gm. per cent, with 3.2 gm. per cent of albumen and 2.1 gm. per cent of globulin. The serum gave a 2 plus cephalin flocculation test in 48 hours; thymol turbidity, 2 Maclagan units; prothrombin time, 60 per cent of normal; cholesterol, 266 mg. per cent; cholesterol esters, 175 mg. per cent; serum bilirubin, 0.25 mg. per cent. Hemoglobin was 14 gm. per cent; red blood cell count, 4.5 millions; white blood cell count, 7,000, with a normal differential count. In one 24-hour urine specimen (1200 cc.) there was 850 mg. of alpha-amino acid.

The clinical course was that of an intermittent disease but neurologic symptoms progressed in severity with each exacerbation. There was no clinical evidence of progressive liver failure. Terminally, the patient lapsed into a coma and expired approximately 2 years after the first symptoms.

The patient had six siblings, one of whom (a girl) died suddenly at the age of 15. There was no history of a previous illness or neurologic symptoms. A limited necropsy revealed nodular cirrhosis of the liver. The brain was not examined. There was no other pertinent family history. The other siblings are living and well at the present time.

Necropsy Findings

At necropsy, the liver weighed 1650 gm. and was diffusely nodular. Microscopically, the architecture was distorted by periportal fibrous tissue. The hepatic lobules were irregular in shape and size and the parenchymal cords had lost their radial arrangement. There was minimal increase in the number of hepatic ducts and round cells in the portal areas. Many of the parenchymal cells were coarsely granular. There were many small, nodular areas of regenerating parenchyma. The remaining thoracic and abdominal viscera were not remarkable. The brain weighed 1260 gm. Coronal sections revealed bilateral small areas of softening in the anteromedial portion of the putamen but no gross cavitation. There was slight, diffuse, reddish brown discoloration of the anteromedial tip of the right globus pallidus. Microscopically, there was minimal diffuse increase in glial cells in the cerebrum and cerebellar cortex. In the globus pallidus, putamen, and caudate nucleus there were small focal areas of encephalomalacia, ranging from early disruption of the architecture to loss of brain substance. Ganglion cells were in various stages of degeneration and there was an increase in glial cells. A few, large, Alzheimer cells were seen. Calcification of small blood vessels of the basal ganglia was present in some areas.

Methods of Examination

Tissues were fixed in neutral 10 per cent formalin, embedded in paraffin, and duplicate sections, 5 μ in thickness, were prepared. One set was stained with hematoxylin and eosin and the other with Mallory's[10] fresh hematoxylin stain for copper and iron. The latter stain was used because it is highly sensitive for copper and easily prepared. Tissues which showed pathologic change, or were stained by fresh hematoxylin, were then studied for copper, as will be described.

One set was treated with rubeanic acid (dithio-oxamide) according to the method described by Okamoto and Utamura.[11] In the presence of copper an insoluble precipitate is formed which has an olive-green color. This is a very specific and highly sensitive method for demonstrating copper.

Slides of the next set were stained with Mallory's fresh hematoxylin stain for copper and iron. This gives a sharp differentiation between iron and copper in formalin-fixed tissue. Copper has a deep blue color, and iron compounds a yellow or yellow-brown color.

Another set of slides was prepared according to the histochemical method of Okamoto and Utamura,[11] using diphenylcarbohydrazide to produce a complex copper compound which has a red-brown to violet color. This test is not specific for copper because, according to the authors, the same colored inner complex is formed also with silver, mercury, lead, cadmium, nickel, and cobalt. It is reasonable to assume that these interfering metallic ions were not present in tissue from this case.

The last set of slides was prepared by a modification of the Waterhouse[12] method for studying copper in fresh tissue. The modification was omission of hydrochloric acid as a penetrating agent. The penetrating agent was not necessary since sections of tissue 5 μ in thickness were used. Diethyldithiocarbamate forms an insoluble precipitate with copper, which has a yellow or yellow brown color. This is a sensitive and specific reagent for copper.

In addition to studying the tissues by histochemical methods, various areas from the brain and liver were analyzed quantitatively by wet ashing the dried tissues and employing diethyldithiocarbamate to develop a color for photometric measurement.

Results

Each histochemical method used to demonstrate copper in animal tissue has certain limitations. For this reason, four methods were

used to lessen the possibility of false-positive results. Tissues which are reported to contain copper have been found positive by all four methods described.

Copper occurred as fine granules in the cytoplasm of regenerating hepatic parenchymal cells and as coarse granules in degenerating cells. Small amounts of copper were found in von Kupffer cells, and in macrophages in the periportal areas. No copper was found in or about small blood vessels of the liver. In the putamen, globus pallidus, and caudate nucleus there were moderate concentrations of copper in the adventitia and media of the small blood vessels and heavy concentration about capillaries. No copper was demonstrated in degenerating ganglion cells. In areas of encephalomalacia, there were small, globular masses which measured 20 to 30 μ in diameter. These were rich in copper.

The spleen was the only other organ in which discrete granules of copper were found. These granules were demonstrated in the reticuloendothelial cells of the sinusoids.

Quantitative determinations of copper in areas in the brain and liver are recorded in Table I. The results are in accord with Cummings'[9] figures (1948) for his cases of hepatolenticular degeneration. The normals quoted in Table I are from his work.

TABLE I

Copper Content of Brain and Liver (Reported as Mg./100 Gm. Dry Tissue)

Tissue	Case reported	Average normal
Cortical white matter	19.0	3.3
Cortical gray matter	44.4	6.2
Cerebellum	36.4	——
Putamen	27.7	9.3
Liver	47.3	10.7

DISCUSSION

It is not always possible to demonstrate copper by histochemical means in human tissue even though quantitative analysis may reveal large amounts present. The reason for this is not understood completely. This fact is apparent here. Quantitatively, the concentrations of copper in the cortical gray substance and liver are approximately equal. Histochemically, copper was demonstrated in the liver but not in the gray substance.

Most of the demonstrable copper in the brain closely surrounded capillaries in the form of small granules. Also, the metal occurred in the adventitia and media of small vessels. In the basal ganglia, calcium, iron, and copper usually were found occurring together as shown by positive Turnbull's blue and alizarinsulfonic acid stains. In contrast, copper in the liver did not occur in or about blood vessels and was not associated demonstrably with either iron or calcium.

Apparently some copper is phagocytized from the blood stream by the reticulo-endothelial cells, as granules were demonstrated in von Kupffer cells, periportal macrophages, and in the lining cells of the splenic sinusoids.

Unfortunately the Kayser-Fleischer ring in the cornea could not be removed for study due to limitation by the necropsy permit.

Summary

After a brief historical review of hepatolenticular degeneration, a case is reported in which the histologic location of copper was studied by four histochemical methods, and quantitative determinations of copper were made on various areas of the brain and liver.

Copper was demonstrated in degenerating and regenerating hepatic parenchymal cells, in reticulo-endothelial cells of the spleen and liver, and in the walls of small blood vessels and about capillaries in the putamen, caudate nucleus, and globus pallidus.

I am grateful to Dr. Morton F. Mason, Professor of Pathological Chemistry in the Southwestern Medical School of the University of Texas, for the quantitative analyses of copper in the tissues.

REFERENCES

1. Rumpel, A. Über das Wesen und die Bedeutung der Leberveränderungen und der Pigmentierungen bei den damit verbundenen Fällen von Pseudosklerose, zugleich ein Beitrag zur Lehre von der Pseudosklerose (Westphal-Strümpell). *Deutsche Ztschr. f. Nervenh.*, 1913, 49, 54–73.
2. Westphal, C. Ueber eine dem Bilde der cerebrospinalen grauen Degeneration ähnliche Erkrankung des centralen Nervensystems ohne anatomischen Befund, nebst einigen Bemerkungen über paradoxe Contraction. *Arch. f. Psychiat.*, 1883, 14, 87–134.
3. Strümpell, A. Ueber die Westphal'sche Pseudosklerose und über diffuse Hirnsklerose, insbesondere bei kindern. *Deutsche Ztschr. f. Nervenh.*, 1898, 12, 115–149.
4. Wilson, S. A. K. Progressive lenticular degeneration: a familial nervous disease associated with cirrhosis of the liver. *Brain*, 1911–12, 34, 295–509.
5. Spielmeyer, W. Die histopathologische Zusammengehörigkeit der Wilsonschen Krankheit und der Pseudosklerose. *Ztschr. f. d. ges. Neurol. u. Psychiat.*, 1920, 57, 312–351.
6. Hall, H. C. La dégénérescence hépato-lenticulaire (maladie de Wilson-pseudosclérose). Masson et Cie, Paris, 1921, 361 pp.
7. Haurowitz, F. Über eine Anomalie des Kupferstoffweschsels. *Ztschr. f. physiol. Chem.*, 1930, 190, 72–74.
8. Glazebrook, A. J. Wilson's disease. *Edinburgh M. J.*, 1945, 52, 83–87.
9. Cumings, J. N. The copper and iron content of brain and liver in the normal and in hepato-lenticular degeneration. *Brain*, 1948, 71, 410–415.

10. Mallory, F. B. Pathological Technique. W. B. Saunders Co., Philadelphia, 1938, p. 139.

11. Okamoto, K., and Utamura, M. Biologische Untersuchungen des Kupfers. I. Über die histochemische Kupfernachweismethode. *Acta scholae med. univ. imp. in Kioto,* 1937–38, 20, 573–580.

12. Waterhouse, D. F. Studies of the physiology and toxicology of blowflies. A histochemical examination of the distribution of copper in *Lucilia cuprina.* Council for Scientific and Industrial Research, Commonwealth of Australia, Melbourne, 1945, Bull. No. 191, 20 pp.

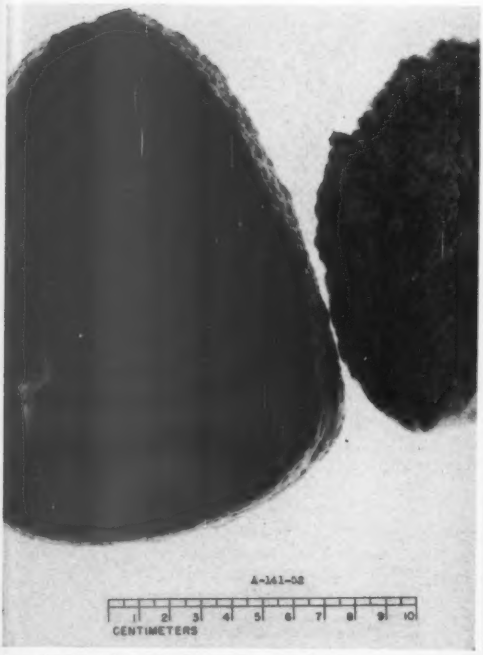
LEGENDS FOR FIGURES

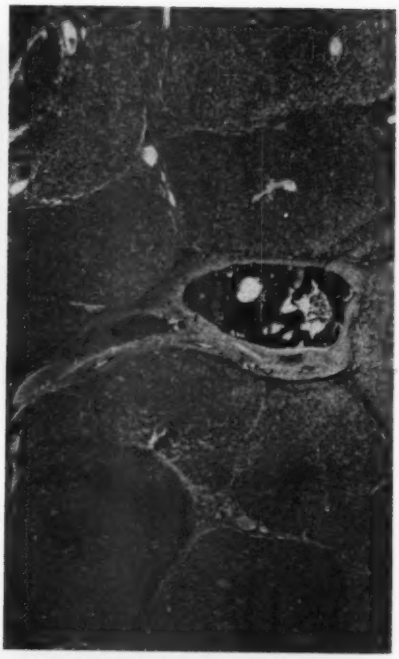
FIG. 1. Cross section and capsular surface of the unfixed cirrhotic liver from the reported case of hepatolenticular degeneration.

FIG. 2. Microscopic section from the gross specimen shown in Figure 1. Hematoxylin and eosin stain. × 60.

FIG. 3. Right basal ganglia from the same case as that from which Figure 1 was taken. The small, black, globular deposits and the dark granular material in the small blood vessel wall contain demonstrable amounts of copper by histochemical methods. This is shown in Figures 4 to 11. Hematoxylin and eosin stain. × 350.

1





2

3

Figs. 4 to 11. All photomicrographs are made from tissue fixed in 10 per cent neutral formalin, embedded in paraffin, and cut 5 μ in thickness.

FIG. 4. Putamen. Modification of Waterhouse's histochemical method for demonstrating copper in fresh tissue. Copper appears as black granules in media and adventitia. (Yellow in true color.) × 150.

FIG. 5. Spleen. Mallory's "copper and iron" stain using fresh hematoxylin. Copper appears as black granules in reticulo-endothelial cells. (Deep blue in true color.) × 480.

FIG. 6. Putamen. Histochemical method of demonstrating copper in tissue using rubeanic acid, after Okamoto and Utamura. Copper appears as gray-black perivascular beads. (Greenish yellow in true color.) × 350.

FIG. 7. Liver. Same method as that for Figure 6. Copper appears as black granules in a liver lobule. (Greenish yellow in true color.) × 350.

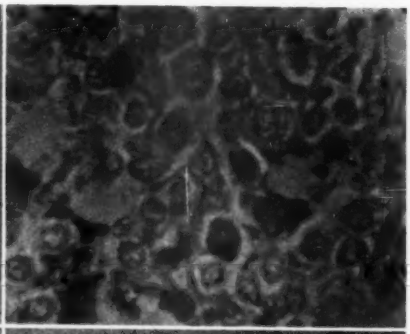
FIG. 8. Liver. Mallory's "copper and iron stain" using fresh hematoxylin. Copper appears as black granules in parenchymal cells. (Deep blue in true color.) × 350.

FIG. 9. Putamen. Same method as that for Figure 8. Copper appears as black granules in media and adventitia of small vessels. (Deep blue in true color.) × 100.

FIG. 10. Liver. Histochemical method for demonstrating copper in tissue using diphenylcarbohydrazide, after Okamoto and Utamura. Copper appears as black granules in a liver lobule. (Reddish brown in true color.) × 250.

FIG. 11. Putamen. Same method as that for Figure 10. Copper appears as black granules in media and adventitia of small vessel. (Reddish brown in true color.) × 250.

4



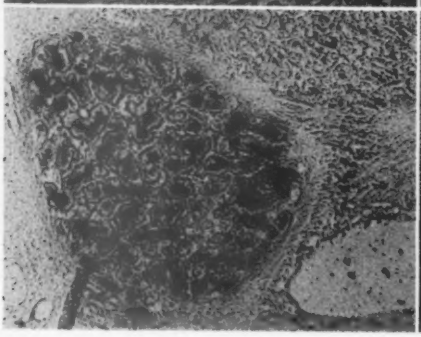
5

6

7

8

9



10

11

FIFTY-SECOND ANNUAL MEETING
OF THE
AMERICAN ASSOCIATION OF PATHOLOGISTS
AND BACTERIOLOGISTS

HOUSTON, TEXAS

APRIL 7TH, 8TH, AND 9TH, 1955

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Fifty-second Annual Meeting
HOTEL SHAMROCK
Houston, Texas
April 7th, 8th, and 9th, 1955

PRESIDENT DUFF IN THE CHAIR

BUSINESS MEETING
April 9, 1955

The following nominations for elective officers were submitted by the Council:

President	DR. EDWIN W. SCHULTZ
Vice-President	DR. GRANVILLE A. BENNETT
Secretary	DR. EDWARD A. GALL
Treasurer	BRIG. GEN. ELBERT DECOURSEY
Incoming Member of Council	DR. DOUGLAS H. SPRUNT

Additional nominations were called for. None having been offered, it was moved and seconded from the floor that the Secretary be instructed to cast a unanimous ballot for the entire slate.

At the direction of the President, the Secretary reported the following actions of the Council:

Election of New Members

Javier Arias-Stella, New York, N.Y.
Melvin B. Black, San Francisco, Calif.
J. M. B. Bloodworth, Jr., Columbus, Ohio
Herbert Braunstein, Cincinnati, Ohio
John H. Childers, Galveston, Texas
Clyde J. Dawe, Rochester, Minn.
Kenneth Martin Earle, Galveston, Texas
Ethel E. Erickson, Houston, Texas
Joseph David Feldman, Pittsburgh, Pa.
Bernard F. Fetter, Durham, N.C.
Lawrence William Gardner, Detroit, Mich.
John Borden Graham, Chapel Hill, N.C.
G. Gordon Hadley, Los Angeles, Calif.
Paul Noel Harris, Indianapolis, Ind.

Leopold G. Koss, New York, N.Y.
Paul Kotin, Los Angeles, Calif.
Leo Krainer, Washington, D.C.
William Ellsworth Loring, Chapel Hill, N. C.
Arthur E. Rappoport, Youngstown, Ohio
Leopold Reiner, Boston, Mass.
Alexander C. Ritchie, Montreal, Que.
E. Stanfield Rogers, Durham, N.C.
Dale M. Schulz, Indianapolis, Ind.
Robert Edward Scully, Boston, Mass.
John Lawton Shapiro, Nashville, Tenn.
Louis-Charles Simard, Montreal, Que.
Harry H. Stumpf, New York, N.Y.
George William Thoma, Richmond, Va.
Abe Towbin, New York, N.Y.
William Oliver Umiker, Ann Arbor, Mich.
Frank Vellios, Indianapolis, Ind.

Acceptance, with regret, of the resignations of Walter L. Bierring, Herbert C. Clark, Abbie I. Knowlton, Robert A. Lambert, Alfred M. Lucas, Frederic Parker, Jr., George L. Rohdenburg, Elizabeth M. Smadel, and William Thalhimer.

With deep regret, the recording of the deaths of Oswald T. Avery, Donald C. Beaver, Paul Brindley, H. Hays Bullard, Henry Bunting, Zola K. Cooper, Virgil H. Cornell, Timothy Leary, Balduin Lucké, A. M. Pappenheimer, M. D. Peñas, Monroe J. Schlesinger, James S. Simmons, Joseph Victor, and Augustus B. Wadsworth.

The re-election of Dr. Shields Warren to the Editorial Board of *The American Journal of Pathology* for a period of six years, beginning January 1, 1956.

The re-election of Miss Dorothy E. Seiferlein as Editorial Assistant of *The American Journal of Pathology* for the ensuing year.

The re-election of Dr. David G. Freiman as Assistant Secretary of the Association for the ensuing year.

The re-election of Dr. Elson B. Helwig as Assistant Treasurer of the Association for the ensuing year.

The Secretary announced that the next annual meeting of the Association will be held in Cincinnati, Ohio, on April 26, 27, and 28, 1956. The topic for the symposium is "Virus Disease."

The Secretary further announced that the annual meeting in 1957 will be held in Washington, D.C., on April 11, 12, and 13.

The President then asked if there was any business from the floor. None was presented, and the business meeting adjourned.

Edward A. Gall, *Secretary*

REPORT OF THE TREASURER

The report of the Treasurer was submitted to the Council and accepted. It was accompanied by a letter of certification from John F. Norris, Accountant, of Silver Spring, Maryland. In condensed form, the Treasurer's report follows:

General Checking Account

Receipts

Balance on hand, January 1, 1954			$ 5,163.90
Membership dues		$ 9,342.33	
Interest on bonds		500.00	
			9,842.33
			$15,006.23

Disbursements

American Journal of Pathology		$ 7,072.00	
Secretary's office, clerical	$300.00		
Printing, travel, supplies	745.65		
Abstracts, clerical	45.50		
		1,091.15	
Treasurer's office, clerical	$150.00		
Stationery, printing, general expense	168.97		
Safety box	6.00		
		324.97	
Miscellaneous			
Int. Assn. Med. Museums	$180.90		
Nat. Soc. for Med. Research	50.00		
Refund of dues	10.00		
		240.90	
			8,729.02
Balance on hand, December 31, 1954			$ 6,277.21

Investment Account

U.S. Treasury bonds, series G, due January, 1957			$20,000.00
Savings Accounts			
Riggs National Bank		$ 6,254.47	
National Bank of Washington		8,591.86	
			14,846.33
Earned but unrealized interest			357.38
			$35,203.71

Elbert DeCoursey, *Treasurer*

SCIENTIFIC PROCEEDINGS

Abstracts

HISTOPATHOLOGIC STUDY OF THE SMALL INTESTINE OF IMMATURE NON-IMMUNIZED MICE INFECTED WITH TRICHINELLA SPIRALIS. John E. Larsh, Jr., William B. Jeffries, and George J. Race (all by invitation), University of North Carolina, Chapel Hill, N.C.

Previous studies have suggested that immunity to *Trichinella spiralis* during the intestinal phase of parasitization is the result (1) of the actions of specific antibodies against the parasite; and (2) of a localized cellular inflammatory reaction which develops at the point of invasion. In immunized mice, there develops a severe acute inflammatory reaction in the upper small intestine at the site of parasitic localization. Following this, the worms are rapidly eliminated from the intestine. In adult immunized mice, the zenith of the inflammatory reaction is reached at 4 days, while in the adult non-immunized mice, the peak of the inflammatory reaction and the elimination of worms is not reached until the eighth day. A low titer of anti-parasitic antibody is demonstrable before the evacuation of the worms from the body. In the current study, non-immunized immature 5-weeks-old mice were infected with 300 *T. spiralis* larvae each. The worm counts indicated that most of the larvae were localized in the anterior small intestine during the initial phase and were expelled from the region only after a marked local inflammation developed within the intestinal wall. The acute inflammatory response in non-immunized young mice reached a peak about the 11th day and persisted through the 21st day. The inflammatory exudate contained predominantly polymorphonuclear leukocytes during the early phase but later contained many plasma cells, lymphocytes, and histiocytes. The worms were expelled from the intestine from the 15th to the 17th day. The results suggest that immature young mice acquire immunity and resistance to *T. spiralis* less readily than do adult mice, and that the localization of *T. spiralis* in the anterior small intestine depends on factors other than the mechanics of peristalsis. These factors are thought to include the development of a specific humoral immunity and a localized inflammatory reaction in the intestine which tend to immobilize the parasite and lead to its elimination.

EFFECT OF FEEDING IRRADIATED TRICHINELLA LARVAE ON PRODUCTION OF IMMUNITY TO RE-INFECTION.* S. E. Gould and (by invitation) Henry J. Gomberg, Frank H. Bethell, John B. Villella, and Constance S. Hertz, Wayne County General Hospital, Eloise, Mich.; Wayne University College of Medicine, Detroit, Mich., and University of Michigan, Ann Arbor, Mich.

Evidence of immunity to re-infection with non-irradiated larvae of *Trichinella spiralis* was found in white rats initially infected with larvae that had been exposed to 10,000 r. gamma rays of cobalt-60 (a dose of radiation that does not prevent the larvae from developing into adult forms but does result in partial or complete sexual sterilization of the adult worms). However, rats that are initially fed larvae exposed to 18,000 r. cobalt-60 (a dose that prevents most larvae from maturing to adult forms) develop little or no immunity as determined by a challenging dose of non-irradiated larvae.

* This article will appear in a subsequent issue of *The American Journal of Pathology*.

MORPHOLOGIC EVIDENCE OF THE HYPERSENSITIVE PATHOGENESIS OF THE LESIONS OF EXPERIMENTAL HYPERSENSITIVITY AND THE COLLAGEN DISEASES. Robert H. More and (by invitation) Henry Z. Movat, Queen's University, Kingston, Ont.

Fibrinoid alteration of collagen has been considered the common denominator of the collagen diseases. Aegerter and Long noted the involvement of many components of mesenchymal origin and suggested that hypersensitivity may play a rôle in the pathogenesis of these changes. Following antigen injection in experimental animals, Ehrich described the proliferation of mesenchymal cells, including plasma cells in many areas. Before this society in 1950, one of us [R.H.M.] described the invariable presence of a prominent, nondescript, large mononuclear cell response in the lesions of the collagen diseases, hypersensitive states in man, and the lesions of experimental hypersensitivity. The anomaly of an apparently acute destructive lesion of connective tissue eliciting a granulomatous inflammatory response was discussed; and it was stated that if morphologic studies were to lead to a knowledge of the pathogenesis of these diseases, as much attention and effort must be devoted to a study of the mononuclear cell response as to fibrinoid. Elucidation of the nature of this cellular response has been the goal of recent studies. These included serial time observations on tissues of sensitized rabbits. In the interval between antigen injection in the rabbit and the appearance of circulating antibody, proliferation of large mononuclear cells occurred in the hematopoietic tissue and paravascular connective tissue. The latter is the site where later, or after a second massive injection of antigen, a necrotizing arteritis occurs. Staining with methyl green pyronin and Giemsa stain indicated that the mononuclear cells were plasma cells in various phases of maturation. This reaction was considered to represent the morphologic equivalence of antibody formation. This function of plasma cells has been repeatedly demonstrated.

Later, when circulating antibody had appeared, histiocytes proliferated in the myocardium and subendothelial position of the endocardium and in other blood vessels in areas normally abundant in acid mucopolysaccharide. We are of the opinion that this reaction may represent a response to the union of antigen previously deposited in those sites (Coons *et al.*) and the circulating antibody. At a stage when there had been time for the fixation of antibody in the tissues and especially when, at this time, a second massive dose of antigen was given, there developed an acute necrotizing arteritis similar to polyarteritis nodosa. We believe this violent destructive reaction may represent a response of the tissue to the union of fixed tissue antibody and circulating antigen. Plasma cells and their precursors are an intimate and prominent part of these lesions. Analogous findings, which we believe justify a similar interpretation, have been found in cases of sulfonamide hypersensitivity, acute disseminated lupus erythematosus, polyarteritis nodosa, and dermatomyositis. In these cases plasma cells and their precursors were a prominent feature of the destructive connective tissue lesion.

The proliferation of antibody-forming cells immediately after antigen injection at sites where fibrinoid alteration later developed indicates that the systemic destructive necrotizing lesions in experimental hypersensitivity are related pathogenetically to the immune responses of the body. Furthermore, the demonstration of plasma cells in similar lesions in the collagen diseases provides direct evidence that the destructive lesions of these diseases are causally related to some aspect of local immune phenomena.

EFFECT OF HYALURONIDASE ANTAGONISTS ON THE REACTION OF THE HOST TO VIRAL INFECTION. John M. Pearce, New York Hospital—Cornell University Medical Center, New York, N.Y.

Rabbits inoculated intracutaneously with fibroma virus, vaccinia, and myxoma virus and simultaneously injected with antihyaluronidase substances at the same

site react differently to local inoculation than do those which have received virus alone. With fibroma virus the usually resulting tumor can be completely inhibited or markedly decreased in size. The vaccinia lesion makes itself evident after a longer period of time and is smaller. Animals that recover from these two infections are immune to further inoculation. The acquisition of immunity to fibroma virus occurs even when complete inhibition of tumor formation has resulted from the combination of virus and antihyaluronidase injection. This is an effect of the hyaluronidase antagonist on the tissues of the host rather than on the virus as is shown by the neutralizing effect of added hyaluronidase, which releases the virus activity. The local lesion produced by the intracutaneous inoculation of the myxoma virus may be delayed in its appearance and made smaller in size than in control animals but, as yet, generalization of the infection and the ultimate death of the animal has always occurred.

PANCREATIC LESIONS AND PERIPANCREATIC FAT NECROSIS IN CORTISONE-TREATED RABBITS. Harry H. Stumpf (by invitation) and Sigmund L. Wilens, Bellevue Hospital and New York University College of Medicine, New York, N.Y.

Pancreatitis was observed in 21 and peripancreatic fat necrosis in 8 of 27 rabbits injected intramuscularly with 4 to 8 mg. of cortisone daily for 13 to 81 days. Blood amylase was elevated in rabbits with cortisone pancreatitis. The foci of pancreatic fat necrosis were visible on minute gross examination.

The occurrence of pancreatitis in cortisone-treated animals has not been reported hitherto. Although a number of agents, notably ethionine, produce pancreatitis in several species of laboratory animals, such lesions have not been described in the rabbit. The lesion in cortisone-treated rabbits began with focal areas of acinar cell degeneration followed by atrophy and ductular proliferation. Small concretions were found in intralobular ductules and these were considered to be particularly characteristic. Inflammatory changes were mild and secondary except at the margins of fat necroses, where they were similar to those seen in association with human pancreatitis. Fibrosis was present in older lesions but was never pronounced. The acinar damage did not appear to be related to liver injury or to hyperplasia of the islets of Langerhans, although these two changes were often present. There is evidence that cortisone administration increases and perhaps alters the exocrine secretion of acinar cells but the exact mechanism by which cortisone produces pancreatic damage has not been elucidated. It is suggested, however, that these lesions may be related to the marked, cortisone-induced, alimentary type of hyperlipemia. It has been postulated in certain human cases of essential hyperlipemia that the recurrent pancreatitis so often associated with this disorder is a result of the predominantly neutral fat hyperlipemia, rather than its cause. It may by that there is a similar underlying mechanism in these rabbits and man.

INFLUENCE OF CORTISONE ON THE DEVELOPMENT OF THE EXPERIMENTAL SILICA GRANULOMA IN THE RAT. Wallace H. Clark, Jr. (by invitation), Tulane University School of Medicine, New Orleans, La.

Clinical and experimental studies by many workers have demonstrated the anti-inflammatory properties of cortisone. These investigations have employed a variety of injurious agents, including living organisms. The inflammatory response to bacteria is difficult to interpret because the organism itself presents to the host the chemical complexity of a living system. In addition the cellular response of the host is frequently altered by immune or allergic mechanisms developing during the course of a bacterial infection. The influence of cortisone in such complicated inflammatory processes is poorly understood, as it may affect the cellular response, *per se;* or influence antibody production. An injurious agent of known, simple chemical composition, apparently devoid of antigenic properties, permits the study of cortisone

effects on the inflammatory cells themselves. The present studies, using silica, have demonstrated an inhibition by cortisone of macrophage and fibroblastic response, collagen production, and epithelioid cell formation.

Finely divided silica (average particle size, 2.9 μ) was injected in saline suspension into the subcutaneous tissue of the back of male Sprague-Dawley rats. The animals were sacrificed at 1, 2, 4, 8, 16, and 32-day intervals, and the resulting lesions excised, weighed, and studied histologically. In 10 mg. quantities silica produces, during the first 48 hours, an acute inflammation; at 4 days, pronounced fibroplasia; at 8 and 16 days, progressive fibrosis; and at 32 days, in addition to fibrosis, distinct "epithelioid cell tubercles" are formed. If animals are treated with 10 mg. of cortisone daily, beginning 48 hours before the injection of silica, the degree and nature of the granulomatous response is profoundly altered. The early acute response is probably not significantly influenced, although detailed studies of lesions less than 24 hours old have not been made. At 4 days, however, there is striking reduction in the number of fibroblasts and macrophages accumulated at the site of injection of silica. At 8 and 16 days, the number of these cells is greatly diminished as compared with controls and collagen production is minimal. No "epithelioid cell tubercles" develop in 32-day lesions in treated rats. Under the conditions of these experiments, cortisone diminishes macrophage response, fibroplasia, and collagen production, and inhibits "epithelioid cell" formation in experimental silica granulomas.

Preliminary studies show that the lesion can be varied, at least quantitatively, by alteration in the particle size of the silica. When silica of a particle size of 10 to 20 mμ is used, the fibroplasia characteristic of 4-day lesions is so pronounced that it resembles fibrosarcoma histologically and may weigh fifteen times as much as lesions produced with the same amount of silica of an average particle size of 2.9 μ.

SYSTEMIC KARYOMEGALY. CASE REPORT OF A HERETOFORE UNDESCRIBED DISEASE ENTITY. Alan R. Moritz and (by invitation) Cecilie Leuchtenberger, Western Reserve University, Cleveland, Ohio.

The presence of widely distributed nuclear abnormalities was an unexpected finding in the post-mortem examination of a young man who died of chronic progressive pulmonary fibrosis and right heart failure. Monstrous bizarre nuclei were encountered in the epithelial cells of the renal tubules, the adrenal cortex and medulla, the prostate, the exocrine glands of the pancreas, the mucosal glands of the alimentary tract, and irregularly in skeletal muscle cells, neurilemmal cells, and the cells of interstitial fibrous connective tissue. Nuclear volumes of some of the affected cells were increased by as much as thirty times the normal value. Comparable increases, up to twenty times, occurred in the DNA content of these nuclei. The DNA increase had not occurred in multiples of the amount normally present, as would have been the case if the increase had resulted from polyploidy. Neither was there evidence of mitotic division which, if present, might conceivably have accounted for the intermediate values that were observed. The configuration of the DNA masses within the abnormal nuclei was more consistent with that of inclusions than with that of chromatin.

The significance of these nuclear abnormalities was not determined. That the increased bulk of the nuclei was due to an increase in DNA, that the amounts of the increase did not represent multiples of the normal diploid values, and that the configuration of the abnormal DNA masses was similar to that of the nuclear inclusions which occur in viral disease and dissimilar to that of chromatin, suggest virus infestation. The report of this case is presented in the hope that it will lead to the discovery of other cases, with eventual clarification of the cause and nature of the disease.

MUMPS ENCEPHALITIS: PATHOLOGY AND PATHOGENESIS. W. L. Donohue and (by invitation) F. D. Playfair and Lorne Whitaker, Hospital for Sick Children, Toronto, Ont., and St. Catherine's General Hospital, St. Catherine, Ont.

Neurologic complications of mumps which may be designated as meningitis, meningo-encephalitis, or encephalitis are not uncommon. Fatalities are few and even rarer are reports of histologic studies of cases which have been necropsied. Only 4 reports have been found in the literature dating from 1900 which concerned unequivocal cases of acute fatal mumps encephalitis and in which the histologic changes in the central nervous system were even briefly described.

We have had the opportunity to study 2 cases of fatal mumps encephalitis. Both cases were typical clinically. One was a 5-year-old female who developed neurologic signs 7 days after parotitis and died 4 days later. The other was a boy of 14 years who showed signs of encephalitis 6 days after parotitis and succumbed 3 days later. The parotid glands of these patients presented similar lesions. The changes were similar to those which have been described in the few reports which concern human material. The salivary glands exhibited a pronounced periductal inflammatory infiltration with mononuclear cells and lymphocytes. Most of the ducts were distended with conglomerate masses and inspissated secretions, débris, and polymorphonuclear leukocytes. The epithelial lining of the ducts displayed only minor degenerative changes. The histopathologic changes in the central nervous system of both cases were widespread and involved chiefly the white matter. The pre-eminent lesion was an acute perivascular demyelinization similar to that seen in the post-infection type of encephalitis which may follow measles, smallpox, and vaccinia. Neuronal degeneration and necrosis were present to a minor degree. Our findings are in agreement with the 4 unequivocal cases reported in the literature.

In neither of the cases studied was material available for virus study. Although direct proof is lacking, in view of the great advances that have been made recently in our knowledge of infections with the virus of mumps, some circumstantial evidence may be adduced to suggest that the perivascular lesions in mumps encephalitis are not necessarily the result of an "allergic or sensitization phenomena" but may be due to the presence of the virus itself.

NEW APPLICATIONS OF THE LUXOL FAST BLUE MYELIN STAIN. I. A MYELO-ANGIO-CYTO-ARCHITECTONIC METHOD. II. A MYELIN-NEUROGLIA METHOD. III. A MYELIN-FAT METHOD. IV. A "MYELIN"-AXIS CYLINDER METHOD. George Margolis and (by invitation) John Phillip Pickett, Duke University School of Medicine, Durham, N.C.

A study of the Luxol Fast Blue myelin stain developed by Kluver and Barrera has been made. Their observations of the general characteristics of this stain and of its applicability for combined cyto- and myelo-architectonic study of the nervous system have been fully confirmed. Further, it has been demonstrated that this dye may be combined with techniques designed to demonstrate other structural elements in the nervous system. It may be successfully used in conjunction with the periodic acid-Schiff reaction and hematoxylin to provide a myelo-angio-cyto-architectonic picture of exquisite tinctorial quality. In combination with the Mallory phosphotungstic acid-hematoxylin method, a myelin-neuroglia technique which chiefly differentiates these two elements is attained. Used with Sudan IV, a myelin-fat stain of superior clarity is achieved. The dye may also be used as a counterstain following the Holmes axis cylinder method. However, it appears in this procedure that the myelin-holding framework is stained, myelin itself being extracted. These results demonstrate that Luxol Fast Blue has qualities which make possible a broader application of a myelin stain than with any previously developed dye.

STUDY OF CELL GROUPS IN THE HYPOPHYSIS AND THEIR RELATION TO GRANULAR CELL MYOBLASTOMAS. F. E. Davis and E. M. Butt, University of Southern California School of Medicine and Los Angeles County Hospital, Los Angeles, Calif.

Well over 200 cases of granular cell myoblastomas have been reported in mesodermal tissues at the present time. These cell groups have been reported as occurring most frequently in the tongue, with the remainder in such organs as skin, anus, vulva, lacrimal sac, breast, lips, spermatic cord, trachea and bronchi, and the middle ear. Recently, Harland described a lesion of neoplastic proportions in the hypophyseal stalk, which he called a granular cell myoblastoma. The striking histologic resemblance of the granular cells of the peripheral organs and those we have found in the tuber cinereum, hypophyseal stalk, and pars nervosa suggested that these cells are similar in origin and etiology. The neurohypophyseal cell groups in the present study are made up of large cells with small, eccentrically placed, uniformly round nuclei, and pronounced granular eosinophilic cytoplasm. The groups were single and were arranged in fascicles or nests. Histochemically, the granular cells of the neurohypophysis gave the same reactions as those of the granular cell myoblastomas.

Granular cell groups in the neurohypophysis have been studied by numerous authors, first in 1921 by Sternberg, who called them choristomas. Priesel (1922) labeled them progonoblastomas, Shanklin (1947) called them "tumorettes." Harland (1953), apparently the first to recognize their similarity to those of mesodermal structures, called them granular cell myoblastomas.

Since Abrikossoff's (1926) original proposal that these cells were myoblastic in origin, two other hypotheses have been advanced. Leroux and Delarue (1939) thought the cell of origin was a histiocyte storing an unidentified substance. Fust and Custer (1948 and 1949) concluded that these cell groups were of neural origin and implied that the precursor cell is the Schwann cell. Pearse (1950) rejected all three hypotheses (neural, muscular, and histiocytic) of origin. He advanced a fourth hypothesis that the "tumors" are lipid-containing granular-cell fibroblastomas.

We have studied 16 examples of neurohypophyseal granular cells by numerous staining methods and compared them to similarly stained granular cell myoblastomas. The reactions are identical. To our 16 cases we have added 92 cases from the literature. Anatomical site, age incidence, incidence of lesions, and post-mortem findings were analyzed. The etiology of the cell groups in the neurohypophysis seems to be clearer than the origin of the cells composing them. Throughout the course of the supra-optico-hypophyseal tract, as well as at their points of termination, are many neuroglia. Astrocytes are less numerous in these areas while oligodendrocytes are very evident. The functions of the oligodendrocytes are not those of a cohesive nature. Rather, they discharge a function secondary to the fibers' activities. They support or nourish, and take place in the repair following destruction. The oligodendrocyte is the central nervous system homologue of the sheath of Schwann cell of the peripheral nerve. We believe these cell aggregates are an autonomous proliferation of the oligodendrocytes. Further, this proliferation is induced by the disappearance of the neurite or neurites which are in proximity to the oligodendrocytes. The disappearance of the neurite is due to degeneration or destruction of its cell in the nuclei of the paraventricular, supra-optic and tuberis nuclei. Following Pierre Masson's work, an analogy may be drawn. He severed nerves in rabbits and tore out the proximal stumps. These latter were transplanted. In varying periods of time the sheath of Schwann cells underwent a marked proliferation. The neurites had undergone marked degeneration, thereby releasing the Schwann cell proliferation.

Histochemical methods show the cell groups of the present study to be identical with granular cell myoblastomas. These latter are excellent examples of Schwann cell proliferation. Finding granular cell groups in the neurohypophysis where striated

muscle does not exist eliminates the myoblastic theory of origin of granular cell myoblastomas and strengthens the neural concept.

BASIC PATHOLOGIC FORMS OF CEREBRAL PALSY. Abe Towbin (by invitation), Ohio State University, Columbus, Ohio.

In the study of cerebral palsy, the clinical aspects of the disorder have occupied the attention of investigators to an increasing degree. Much less critical attention has been given to the study of the underlying pathologic processes. This is largely due to the difficulty in assembling data of necropsied cases. Because of the heterogeneous nature of the brain lesions in cerebral palsy, interpretation of the causal processes in this disorder has been the subject of much confusion.

In the present investigation, 23 cases of cerebral palsy, studied over a 5-year period, were available for analysis. Three basic pathologic types of cerebral palsy were evident: In the first type the defect in the brain was due to a developmental arrest, hereditary or induced. In most cases of this group, the developmental defect was induced; a history of maternal illness during pregnancy usually was present. At necropsy, true microencephaly was generally a prominent feature. In the most severe example, an 8-year-old child, the brain had the architectural features of that of a 5-months fetus. The second type of cerebral palsy was characterized by encephaloclastic lesions, the result of antecedent processes of systemic nature, such as erythroblastosis fetalis, anoxia neonatorum, and prematurity. Cases related to erythroblastosis fetalis had jaundice and clinical evidence of kernicterus at birth. At necropsy there were destructive lesions in the basal ganglia. The brains in cases related to anoxia usually showed symmetric lobar sclerosis involving the motor cortex; gliotic scars and cystic lesions were prominent. In the third type of cerebral palsy defined in this study, the encephaloclastic lesions were intrinsically of local nature; this group included cases due to mechanical injury of the head, cerebral vascular lesions, and hydrocephalus. Whether of developmental, systemic, or local origin, the lesions in the brain at necropsy were generally of a severity proportional to the neurologic disability noted clinically.

This study stresses the fundamental importance of close correlation of clinical history with necropsy findings in appraising the underlying pathology in cases of cerebral palsy. The findings tend to dispel the deeply ingrained conception that cerebral palsy is commonly due to meningeal hemorrhage associated with mechanical head injury at birth. The importance of systemic disorders as a basis for the pathologic changes in the brain in cerebral palsy are emphasized.

CYSTINOSIS (LIGNAC-FANCONI DISEASE).* Christos D. Gatzimos, Dale M. Schulz, and Raymond L. Newnum (all by invitation), Indiana University School of Medicine, Indianapolis, Ind.

Cystinosis (Lignac-Fanconi disease) is a disease of infants and children characterized by retarded physical development which results in dwarfism, storage of cystine crystals in the cells of the reticulo-endothelial system, and amino-aciduria. Severe rickets is present frequently.

The diagnosis of cystinosis was made in a rachitic dwarf after post-mortem examination. The only pertinent features grossly were the presence of small, yellowish, linear, chalky streaks in the spleen and mesenteric lymph nodes. The kidneys were pale and granular. Histologically, there were vacuolated reticulo-endothelial cells in the spleen, lymph nodes, bone marrow, and liver. The kidneys showed vacuolization of the tubular epithelial cells, interstitial fibrosis, glomerulosclerosis, and arteriolosclerosis. Rachitic changes were present in the bones. Cystine crystals were seen in

* This article will appear in a subsequent issue of *The American Journal of Pathology*.

clumps in the reticulo-endothelial cells of fresh, frozen, or paraffin embedded tissues. The crystals were visible in unstained sections and in sections stained by alcoholic methylene blue. Crystalline material was isolated by aqueous extraction of alcohol-fixed mesenteric lymph nodes. This material was identified as cystine by various chemical tests. Paper electrophoretic studies on this isolated material indicated that only one substance was present in the extract and that this substance showed the electrophoretic behavior of l-cystine. In addition, quantitative determinations of the cystine in the mesenteric lymph nodes were made.

ON THE PATHOGENESIS OF DEATH DUE TO BURNING OF THE SKIN.* Sol Roy Rosenthal and (by invitation) F. Finamore, F. R. Hunter, Alice S. Hunter, and Inza-Nell Roman, University of Illinois College of Medicine, Chicago, Ill.

Earlier studies have shown that burning of dog skin by an *in vitro* method released proteins, mucoproteins, protein split products, histamine, and electrolytes (potassium, sodium, manganese, phosphorus). The amounts varied with the temperature and time of burning.

Using an *in vivo* method in rats that entails injection of air between the skin and the underlying structures and allowing products from burned skin of such a preparation to diffuse into water injected into the air pocket, it was found that concentrates from such diffusates would kill young rats within 2 to 5 hours when injected subcutaneously in amounts of 10 to 12 mg. protein-equivalent per gm. of body weight. In rat preparations in which the skin was separated from the underlying structures for two thirds of the body surface and this followed by immersing portions of the animals in hot water (65° for 30 sec.) corresponding to these areas, death occurred in a higher percentage of such animals than of animals so immersed but not so prepared. Absorption of dye from air pockets beneath burned skin occurred at a higher rate than from similar pockets of non-burned animals. Absorption of C^{14}-labelled "burn toxin" was shown to occur readily by its appearance in the parenchymatous organs when such material was injected into air pockets of normal animals. The circulation of burned skin is non-functioning as was shown by intravenous dye injection experiments. The contribution of the local circulation to the "burn toxin" is discussed. The indications are that the skin plays a major rôle in acute death due to burning, and that there may be a "burn toxin" that originates from the skin.

EFFECTS OF MASSIVE WHOLE BODY RADIATION IN RATS. Simon Koletsky, Institute of Pathology, Western Reserve University, Cleveland, Ohio.

In rats single doses of whole body radiation in the 1,000 r. range uniformly result in death within 4 days. So far, the precise mechanism of death has not been established. The animals develop anorexia, progressive debility, weight loss, and severe diarrhea. Morphologically, the main damage consists of aplasia of bone marrow, marked hyperplasia of lymphoid tissues of the body, and necrotizing lesions of the intestine. In spite of bone marrow destruction and leukopenia, there is little evidence that infection is a significant factor in mortality. Shielding experiments indicate that the intestinal damage plays a more important rôle in the fatal outcome than the injury to hemopoietic tissues. Death appears to be related to physiologic disturbances which have their origin in the intestinal tract.

STUDIES ON HYPERVITAMINOSIS D. Richard H. Follis, Jr., University of Utah College of Medicine, Salt Lake City, Utah.

The administration of acutely toxic amounts of vitamin D to experimental animals (rats and rabbits) leads to profound biochemical and anatomical changes. There is a prompt rise in serum calcium and to a lesser extent in phosphorus con-

* These studies were aided in part by a contract between the Office of Naval Research, Department of the Navy, and the University of Illinois.

centrations. The calcium values reach their peak on the third or fourth day and then fall. The serum phosphorus levels follow a similar pattern. Alkaline phosphatase values begin to fall and low levels are found at the time of death. All these changes are dependent on the age of the animal. An important and as yet unexplained alteration is a rise in pH of whole arterial blood. Values as high as pH 7.8 (at 37° C.) have been observed on the fourth to sixth day of the experiment. There is increasing evidence of renal damage microscopically and by the chemical evidence of rising calcium and phosphorus concentrations in kidney tissue. Concentrations in other tissues, such as myocardium, are greatly elevated. A curious change is found in the bones. This has been designated for some time as "hypervitaminosis-D rickets." There is no change in the calcification of the epiphyseal cartilage; a cessation of growth is found because of the general inanition of the animal. However, wide osteoid borders appear, an indication that osteoblastic activity is continuing and may even be stimulated. The reason for the lack of calcifiability of such osteoid in the presence of increased concentrations of calcium and phosphorus is not clear. The sera of animals poisoned with vitamin D promote calcification of rachitic cartilage *in vitro*.

EFFECT OF LARGE DOSES OF GROWTH HORMONE ON THE PANCREATIC ALPHA CELLS IN THE RAT. Bruno W. Volk and (by invitation) Martin G. Goldner, Jewish Chronic Disease Hospital, Brooklyn, N.Y.

The diabetogenic effect of crude anterior pituitary extract and of purified growth hormone has led repeatedly to studies of the direct action of the hormones of the anterior pituitary lobe on the islets of Langerhans. The experimental results so far are largely contradictory. Because of the considerable importance of the question whether the pancreatic islet cells and particularly the alpha cells are under control of a pituitary hormone, we undertook to study the alpha/beta cell relationship in the pancreatic islets in normal and hypophysectomized rats, as well as in similar hypophysectomized animals treated with large doses of growth hormone over prolonged periods of time. For this purpose Gomori's chrome alum hematoxylin and phloxine procedure as well as a modification of Davenport's silver impregnation method for the visualization of the alpha cells, as elaborated by us, were used. This latter procedure utilizes also Bouin-fixed material and thus permits differential counts in serial sections which can be stained alternately by this and by the Gomori technique. In hypophysectomized rats allowed to survive up to 4½ months after operation, no changes in the islets of Langerhans were observed. The daily subcutaneous administration of 1 mg. per 200 gm. of body weight of purified growth hormone for 3 weeks resulted in hyperplasia of the islets with a significant increase of the alpha/beta cell ratio. The daily administration of similar amounts of growth hormone to hypophysectomized rats for 6 weeks, however, produced an absolute and relative decrease of the alpha cells. Those remaining disclosed extensive degranulation of the cytoplasm. The beta cells of these animals similarly showed degranulation and occasional necrosis. It appears, then, that in the hypophysectomized rat allowed to survive up to 4½ months after removal of the pituitary body, the alpha cells are histologically unchanged and the alpha/beta cell ratio remains normal. On the other hand, the prolonged treatment of hypophysectomized rats with large unphysiologic doses of pituitary growth hormone exerts a significant morphologic effect on the alpha cells and to a lesser degree on the beta cells of the pancreatic islets. These changes are not accompanied by disturbances of blood sugar homeostasis.

HISTOCHEMICAL OBSERVATIONS ON HYALINIZED ISLETS OF LANGERHANS IN DIABETES MELLITUS. Wilfred E. Toreson and James F. Rinehart, University of California School of Medicine, San Francisco, Calif.

Hyalinization of the islets of Langerhans has been regarded variously 1) as a

cause of diabetes, 2) as an effect of diabetes and, 3) as a pathognomonic lesion that is merely an associated phenomenon without etiologic significance. The source and the composition of hyalin have not been determined with certainty. In the present studies hyaline material was demonstrated in the islets in 22 of 50 pancreases of diabetic subjects. In most instances the hyaline deposits appeared to be acid mucopolysaccharides. Mucoprotein or glycoprotein was also present in a few. In 4 cases sulfates were present in the acid mucopolysaccharides. A few collagen fibers were demonstrable together with mucoprotein or glycoprotein in only 3 cases. The method for acid mucopolysaccharide was that developed by Rinehart and Abul-Haj; sulfates were demonstrated by a modification of the Gomori fuchsin-aldehyde technique. The source of the mucopolysaccharides was not determined. However, it is improbable that fibroblastic proliferation and collagenization have any significance in the development of this lesion. It is suggested that the accumulation of mucoid material in the pericapillary space might significantly impair function even in the absence of extensive destruction of beta cells.

GLOMERULAR ULTRAVIOLET ABSORPTIONS AFTER CORTISONE THERAPY, SIMULATING DIABETES MELLITUS. Sheldon C. Sommers, Massachusetts Memorial Hospitals, Boston, Mass.

A previous study of the ultraviolet absorptive properties of human glomeruli with the Polaroid color-translating microscope has shown a characteristic stromal absorption in diabetes mellitus, with, or independent of, associated renal diseases. Color translation of diabetic glomeruli as orange was observed especially at the shorter wave-lengths (248–235 $m\mu$). Investigations of kidney tissues from Cushing's syndrome and non-diabetic persons treated with cortisone have shown a similar abnormality of the glomerular ultraviolet absorptions. After treatment with hyaluronidase, the orange color-translated stromal absorptions became normal. It is suggested that these glomerular abnormalities in human diabetes mellitus are associated with the presence of an abnormal mucoprotein and that endocrine factors are involved in its deposition.

HYPERADRENOCORTICISM: A HISTOCHEMICAL STUDY OF SURGICALLY RESECTED ADRENAL GLANDS. Richard B. Cohen (by invitation), William Chapman (by invitation), and Benjamin Castleman, Massachusetts General Hospital, Boston, Mass.

This paper presents the results of a histologic and histochemical study of the hyperactive adrenal cortex in Cushing's syndrome. The Cushing's cases included 16 pairs of hyperplastic glands and 4 unilateral adenomas. In 2 of the latter cases biopsies of the opposite uninvolved adrenal gland were obtained. As controls, adrenal segments were obtained during retroperitoneal procedures from cases without endocrine stigmas, hypertension, or prolonged chronic disease. To implement those controls, 15 pairs of adrenal glands were obtained at necropsy from patients who died suddenly. Sections were stained with hematoxylin and eosin, Sudan IV, Schultz and Windaus reaction for cholesterol, the Ashbel-Seligman reaction for free carbonyl groups, and the Schiff reaction for aldehydes. Criteria for the morphologic diagnosis of adrenal hyperplasia were described with special emphasis on early changes. It was found that before the hyperplastic glands exceeded an arbitrary normal weight of 12 gm., the fascicular zone widened and impinged on or often obliterated the glomerular and reticular zones. Another early change was the formation of intracortical nodules in the periphery of the fascicular zone. Sharp depletion of lipids with marked reduction of cholesterol in the inner cortical zones was always present. The development of cortical nodules was traced by the use of histochemical techniques and several varieties of cortical nodules were defined and compared with

adenomas. The relationship between diffuse and nodular hyperplasia and adenomas was discussed.

ASSOCIATION OF MASCULINIZING OVARIAN HILUS CELL TUMORS, OVARIAN STROMAL HYPERPLASIA, AND LUTEIN-LIKE CELL PROLIFERATION. William H. Sternberg, Tulane University School of Medicine, New Orleans, La.

The presence of abundant ovarian hilus cells (Leydig cells) in women of the fifth decade and beyond is often associated with a marked degree of ovarian stromal hyperplasia. This association appears especially prominent in some women with masculinizing tumors of the ovary. A sequence of six ovarian hilus cell tumors (two of which have previously been reported) illustrates this apparent relationship. In all the hilus cell tumors, characteristic crystalloids of Reinecke were demonstrated microscopically.

The oldest patient, 86 years of age, had been masculinized since the age of 54. She had marked hilus cell hyperplasia, and a yellow-brown tumor of hilus cells in the mesovarium of each ovary. The ovaries showed a surprising lack of atrophy for a woman of 86 years. Microscopically, the ovarian stroma was hyperplastic and contained numerous foci of lutein-like cells. Ovarian stromal hyperplasia and foci of lutein-like cells were present also in the other masculinized patients with the exception of the youngest member of the series who was 39. One masculinized patient, 68 years old, had remarkably well developed skeletal muscles. In addition to hilus cell hyperplasia and tumor, this patient had prominent lutein-like cell proliferations in the ovarian stroma. She also had large uterine leiomyomas, well preserved muellerian epithelia, endocervical and endometrial polyps, carcinoma *in situ* of the cervix, and carcinoma of the breast. Postmenopausal bleeding, present for several years prior to her pelvic operation, had been cyclic in pattern. The clinical picture suggested overproduction of both androgens and estrogens and, microscopically, ovarian hilus cells and ovarian stroma both appeared active.

The histologic evidence suggests that just as theca cell tumors develop out of a background of ovarian stromal hyperplasia, ovarian hilus cell tumors appear to develop in ovaries that are "stimulated" and show both hilus cell and stromal hyperplasia. This is further exemplified in one tumor of the series in which the involved ovary contained numerous nodules of ovarian hilus cell tumor, especially in the medullary region, and multiple nodules of theca cell tumor in the cortical region. The possibility that some of the lutein-like cell foci seen in ovarian stromal hyperplasia may be a source of androgens in addition to that derived from ovarian hilus cells is to be considered. An additional case of theca cell tumor in a masculinized 17-year-old female suggests that cells derived from ovarian stroma may, at least under conditions of neoplasia, sometimes produce androgens.

ENDOMETRIAL HYPERPLASIA AND CARCINOMA PRODUCED BY ESTROGEN. William A. Meissner and Sheldon C. Sommers, New England Deaconess Hospital, Boston, Mass.

A series of diabetic patients given injections of long-acting estrogens during pregnancy were found to develop florid post-partum endometrial hyperplasia, as reported at the 1949 meeting of this Association. To investigate the basis of these extreme endometrial overgrowths and their relation to cancer, rabbits were injected with stilbestrol in oil and observed for long periods. Other rabbits rendered diabetic with alloxan also were given estrogen injections and similarly studied, with suitable controls. Two of 7 estrogen-treated rabbits and 3 of 9 diabetic rabbits given estrogen developed endometrial carcinomas. There was no morphologic evidence that the alloxan diabetic state influenced the endometrial hyperplasia or neoplasia produced

by estrogen stimulation. The significant differences in the host reactions of groups that did or did not develop cancer were increase in pituitary hypertrophic amphophils and nodular adrenal cortical hyperplasia in the cancer hosts.

POSSIBLE ENDOCRINE SIGNIFICANCE OF HYPOPHYSEAL TUMORS IN MAN. Agnes Burt Russfield (by invitation) and Leopold Reiner (by invitation), Massachusetts General Hospital and Beth Israel Hospital, Boston, Mass.

A comparative study was made of the hypophyses of 7 acromegalic patients, one giant, and 19 patients with so-called chromophobe adenomas. In all of these patients who had received no previous endocrine medication, the cellular composition of the hypophyseal tumors was essentially identical. With the Mallory method, the cytoplasm could be stained variably red, blue, or lavender. Occasional Schiff-positive granules were present. Nuclei were large and vesicular, particularly in the acromegalic patients. These tumors, therefore, appeared to be composed of "amphophils" rather than of true "acidophils" or "chromophobes." Administration of thyroid extract, sex hormones, or adrenal steroids tended to reduce cell size, to further nuclear pyknosis, and to increase the proportion of true acidophils and chromophobes. Review of the clinical and anatomical data with respect to other endocrine glands suggested that gonadal or thyroid failure may have preceded tumor formation in many of these patients. Adrenal hyperplasia was found in many patients both with acromegaly and with "chromophobe adenomas." The hypotheses are advanced, first, that the amphophilic cells may secrete growth hormone, ACTH, thyrotropin, and gonadotropin; and second, that end-organ deficiency may be involved in the pathogenesis of some hypophyseal tumors in man.

HISTOCHEMICAL STUDIES ON THE ACTIVATION OF TISSUE ALKALINE PHOSPHATASE AND 5-NUCLEOTIDASE. David G. Freiman, University of Cincinnati College of Medicine, Cincinnati, Ohio.

Cationic activation of alkaline phosphatase and 5-nucleotidase in acetone-fixed, paraffin-embedded sections of human, rabbit, and guinea-pig tissue was studied following exposure of the sections to ethylenediamine tetraacetic acid sodium (EDTA) at pH 6.0. Under the conditions of the experiment, the chelating agent can compete successfully with the functional groups of the enzymes for the activating metallic components and the soluble chelates can be readily washed free of the tissue. Upon exposure of the inactivated sections along with untreated controls to 0.05 to 0.1 M. concentrations of various metallic cations in acetate buffer at pH ranges of 4.0 to 6.0, the effects of magnesium, calcium, cobalt, and zinc ions were found to be essentially the same for both enzyme systems. There was gradual loss of enzyme binding power for magnesium and calcium as the pH approached 5.0 and less marked loss of cobalt-binding capacity. Only zinc, of the metals tested, proved to be an efficient activator at pH values as low as 4.0. No significant differences in enzyme localization were noted in control and reactivated sections. Nickelous ion proved to be a much more efficient activator of 5-nucleotidase than of alkaline phosphatase in the pH range of 5.0 to 6.0, and cadmium ion showed a similar, though less marked, effect. Most significant was the effect of manganous ion which activated alkaline phosphatase only in the very narrow range of pH 5.5 to 4.9, with no effect above pH 5.6 or below 4.8. 5-Nucleotidase was not activated. The significance of this phenomenon with respect to the nature of the functional groups of this enzyme will be discussed.

The distinctive reactivation patterns of the two enzymes studied indicate significant differences in the nature or configuration of their functional groups and provide a histochemical approach to the problem of differentiation between enzymes with more narrowly overlapping pH ranges and tissue distributions. The chelate stability

of the enzyme relative to EDTA has proved to be a limiting factor, however, as evidenced by the fact that it has not been possible to inactivate tissue acid phosphatase or non-specific esterase by this means.

FACTORS INFLUENCING METACHROMASY IN SOLUTIONS. M. D. Schoenberg (by invitation) and J. L. Orbison, Institute of Pathology, Western Reserve University, Cleveland, Ohio.

The mechanism of the metachromatic reaction between thiazine dyes and several polysaccharides has been studied as a function of ionic strength, temperature, substrate concentration, and di-electric constant. This approach to the problem was suggested by the fact that many previous investigators had demonstrated the sensitivity of metachromasy to the environmental conditions under which the reaction occurred. Evidence collected by the present study indicates that although polar bonding of the dye to the substrate is important for the development of metachromasy, it is not sufficient to produce the color change. The evidence further indicates that, in addition to the polar bonds, secondary forces are necessary to produce the inter-dye relationships required for the metachromatic shift. In fact, it appears that it is this inter-dye bonding that is responsible for the shift. Configurational changes in the polysaccharide substrate as induced by changes in the environment of solution influence both the polar and secondary bondings.

ELECTROPHORETIC ANALYSIS OF AMYLOID. Bernard M. Wagner (by invitation), Hahnemann Medical College and Hospital, Philadelphia, Pa.

By means of filter paper electrophoresis, the protein, lipoprotein, and mucopolysaccharide content of amyloid was studied. Amyloid was obtained from the livers of two cases of amyloidosis immediately at post mortem. These cases were not associated with multiple myeloma or chronic infection. One of the cases presented a nephrotic syndrome clinically. Serum from this patient was available for study. Evidence will be presented that amyloid consists of a globulin-mucopolysaccharide complex and that a variety of amyloids exist. The hypothesis that amyloid represents the tissue corollary of abnormal circulating proteins will be discussed.

SERUM ELPHEROGRAMS IN EXPERIMENTAL NEPHROPATHIES OF GUINEA-PIGS. O. J. Pollak and (by invitation) P. T. Cruz, Kent General Hospital, Dover, Del.

This study was made in an attempt to facilitate differential diagnosis of nephropathies. Unilateral and bilateral hydronephrosis, pyonephrosis, pyelonephritis, toxic nephrosis, renal amyloidosis, and calcinosis were induced in guinea-pigs. Blood was studied chemically and by paper electrophoretic fractionation of proteins, lipoproteins, and glucoproteins.

The serum of normal guinea-pigs differs from that of man: Its total protein is lower, alpha$_2$-globulin higher, cholesterol lower, total lipoproteins lower, and alpha$_2$-glucoprotein higher. In all guinea-pigs with induced nephropathy, serum total protein remained rather stable while albumin decreased and globulin increased. Rise in globulin was mainly due to alpha$_2$-globulin. The decline of albumin was most severe after parathormone injections. Ribonucleate injections caused, in addition to the other changes, increase in gamma-globulin. In all animals there was a moderate hypercholesteremia and marked increase in total lipoproteins. Albumin-alpha$_1$-lipoprotein decreased and fast migrating beta-fractions increased while slow moving beta-lipoprotein fractions decreased. In all experimental animals there was an increase in glucoproteins. As a rule, the albumin-glucoprotein declined and alpha$_2$-glucoprotein rose. These deviations were most severe on injection of ribonucleate. However, animals treated with parathormone reacted with an increase in albumin-glucoprotein and the rise in alpha$_2$-glucoprotein was but moderate.

QUALITATIVE ANALYSES OF ANTIGENS OF MYELOMA SERUMS WITH THE AGAR-GEL TECHNIQUE. W. G. Rice, St. Louis School of Medicine, St. Louis, Mo.

Ouchterlony's agar-gel method for the qualitative analysis of serum antigens was used to study the differences in antigens in normal and in six myeloma serums. Examples of the so-called gamma, beta, and alpha types as shown by filter paper electrophoresis were included. Adult rabbits were immunized to normal and myeloma serums, using Freund's adjuvant procedure. In addition rabbits were immunized to the serum from a case of Waldenström's macroglobulinemia and to that from a case of idiopathic agammaglobulinemia. The patterns of the numerous antigen-antibody lines were studied in relation to each other, using the agar-gel technique. In one instance of myeloma, a heavy cryoglobulin precipitate was washed free of other serum components. Serum from rabbits immunized to this relatively pure myeloma globulin were studied in relationship to the various myeloma and other serums. Myeloma globulins have inter-related specificity, but apparently also have distinctive antigenic properties as shown by the anti-cryoglobulin serum.

PATHOPHYSIOLOGIC EVALUATION IN DOGS OF TWO SURGICAL PROCEDURES USED IN PATIENTS WITH OCCLUSIVE CORONARY ARTERY DISEASE. R. Maniglia (by invitation) and Alvin A. Bakst (by invitation), Hahnemann Medical College and Hospital, Philadelphia, Pa., and Jewish Hospital, Brooklyn, N.Y.

Implantation of the left internal mammary artery in the wall of the left ventricle (Vineberg operation) and arterialization of the coronary sinus in two stages (Beck operation) have been advanced as surgical procedures aimed at restoring an adequate supply of arterial blood to the myocardium in cases that have occlusive coronary artery disease.

In dogs with the left internal mammary artery implanted in the left ventricle, the implanted portion of the internal mammary artery underwent progressive luminal obliteration by means of intimal proliferation of collagen and elastic fibers, in some cases with superimposed organized thrombi. The artery was invested with a dense acellular collagenous cuff in which there was minimal vascularization. That these vessels were not capable of delivering adequate blood to the myocardium was borne out by physiologic studies on blood flowing in a retrograde direction from a cannulated circumflex coronary artery.

In dogs with arterialized coronary sinuses, followed up to 1 year, the coronary sinus and large veins located on the anterior, lateral, and posterior surfaces of the left ventricle underwent luminal obliteration through intimal proliferation of collagen and elastic fibers with frequent superimposed organized thrombi. That this occlusive process interfered with the retrograde flow of blood through the venous channels was borne out by physiologic studies on the blood obtained from a cannulated circumflex coronary artery.

With time, both operations fail to accomplish their purpose in dogs because of progressive occlusion of the vascular channels that were intended to restore an adequate supply of arterial blood to the myocardium.

RETROCARDIAC, INTERCORONARY, AND TRANSPLEURAL COMPONENTS IN EXPERIMENTALLY PRODUCED COLLATERAL CIRCULATION TO THE HEART. J. L. Kline, W. E. Bloomer, H. Stern (all by invitation), and A. A. Liebow, Yale University School of Medicine, New Haven, Conn.

It has been demonstrated in this laboratory that cardiopneumonopexy after ligature of the pulmonary artery in the dog results in the establishment of precapillary transpleural connections between branches of the coronary and expanded bronchial arteries. More recent observations, over intervals as long as 1 year following the operative procedure, have shown that these coronary-bronchial arterial anastomoses

not only remain patent, but enlarge, to exceed a diameter of 1 mm. in some instances. With the use of plastic casting techniques, it has also become evident that intercoronary connections, and greatly enlarged branches from the retrocardiac plexus, supplement the collateral circulation. This becomes sufficient to maintain the patency of the anterior descending branch of the left coronary artery after ligature performed several months following the cardiopneumonopexy. Enlargement of the extracoronary retrocardiac plexus, which normally supplies the wall of the left atrium and interatrial septum in the dog, appears to be consequent to expansion of the bronchial arteries with which they have a common origin.

SODIUM AND POTASSIUM CONTENT OF RABBIT PLASMA AND ERYTHROCYTES IN RELATION TO AGE AND TO BLOOD PRESSURE. Douglas Waugh and (by invitation) James R. Stuart, Pathological Institute, McGill University, Montreal, Que.

A study was undertaken to determine whether there is any consistent change in intracellular and extracellular concentrations of sodium and potassium in the blood of rabbits of different ages. Blood pressure was determined in intact animals and in those subjected to renal compression as a means of inducing hypertension. The results of the experiments make it possible to relate sodium and potassium concentrations (or the ratio of these ions to one another) to spontaneous age-period fluctuations in blood pressure and to renal hypertension. The results indicate that plasma concentrations of sodium and potassium are relatively constant between the ages of 4 or 5 months and 4 years. With increasing age, however, there is progressive and significant lowering of intracellular potassium in the erythrocytes, associated with a parallel rise in sodium. Blood pressure observations indicate that between the ages of 3 and 10 months the rise of the intracellular sodium:potassium ratio is accompanied by a progressive and significant rise in blood pressure. After the age of 15 months, however, the mean blood pressure of the different groups of animals remained relatively constant in spite of a continuing rise of the erythrocyte sodium: potassium ratio. In other experiments young animals were subjected to renal compression, following which hypertension developed in most of them. Blood pressure elevation in these animals was not accompanied by any constant shift of the intracellular sodium:potassium ratio.

It is concluded that although a rise of the erythrocyte sodium:potassium ratio accompanies spontaneous blood pressure elevation in young rabbits, the ratio continues to rise independent of blood pressure change in older animals. It is not consistently abnormal in those with renal hypertension. The fact that young and old animals produce erythrocytes containing different proportions of sodium and potassium appears therefore to have no immediate relation to age-period changes in blood pressure.

CORONARY ARTERIOSCLEROTIC HEART DISEASE IN THE YOUNGER AGE GROUP; ITS GREATER FREQUENCY AMONG AN INCREASINGLY OLDER NECROPSY POPULATION. Otto Saphir and (by invitation) Leonard Ohringer, Michael Reese Hospital, Chicago, Ill.

Arteriosclerotic heart disease in the younger age group is not rare. However, as compared to previous years a significant increase in its occurrence was noted recently among our necropsy population in patients under 50 years of age, the percentage increasing from 7.3 to 12.2. The over-all incidence of arteriosclerotic heart disease, however, remained the same, being about 31 per cent of the total number of necropsies. During the same period there was also a distinct decline in the number of necropsies on patients with arteriosclerotic heart disease over the age of 50, the percentage decreasing from 46.6 to 29.1 in this age group. On the other hand, the age distribution of *all* adult necropsied patients has changed as illustrated by the fact that the proportion of necropsies in individuals under 50 years of age has dropped

from 38.3 to 12.9 per cent. Possible factors for the increase in arteriosclerotic heart disease in the younger age group and for the shift in the ages of our necropsy population were discussed.

PATHOLOGIC AND PHYSIOLOGIC EFFECTS OF HEMORRHAGIC SHOCK ON THE HEART. Donald B. Hackel, Western Reserve University School of Medicine at City Hospital, Cleveland, Ohio.

The rôle of the heart in the terminal circulatory collapse of hemorrhagic shock was studied in 19 intact dogs by pathologic, physiologic, and biochemical techniques. The dogs were heparinized and maintained at a mean arterial blood pressure of 30 mm. of Hg for 60 minutes. Three dogs died during the shock period and 6 dogs were reinfused with blood after the 60 minutes of shock. Four of these 6 dogs died without maintaining a normal arterial pressure for more than a few hours. Six of the 7 animals that died showed slight to marked hemorrhage limited almost entirely to the subendocardial region of the left ventricle. The 2 surviving dogs were sacrificed after 5 and 14 days, and showed foci of fatty degeneration, necrosis, and chronic inflammation in a similar subendocardial location. Six additional dogs were given an intravenous drip of nor-epinephrine after 60 minutes of shock and were then infused with blood. Six of these dogs died without maintaining a normal blood pressure and 4 were sacrificed in 7 to 14 days. The pathologic changes in this group were similar to those previously described.

Physiologic and biochemical measurements were made also in all dogs before, during, and after the period of shock, including coronary blood flow (N_2O method), cardiac output (Fick); cardiac work; cardiac efficiency and cardiac utilization of oxygen, glucose, lactate, and pyruvate. Electrocardiograms were recorded. The results of these measurements were interpreted as indicating relative myocardial insufficiency of oxygen during shock, with an enzymatic block involving the myocardial metabolism of carbohydrate. Since the effects of myocardial anoxia are most pronounced in the subendocardial region of the left ventricle, it is reasonable that this is the explanation for the pathologic changes in this location.

ANEURYSMS OF THE AORTA: A REPORT OF 358 CASES. Paul Brindley* and Vernie A. Stembridge, University of Texas Medical Branch, Galveston, Texas.

A review of 9,300 consecutive necropsies performed at the University of Texas Medical Branch Hospitals during a 60-year period from 1892 to 1953 showed 358 cases to have 401 aneurysms. Because of the increasing interest in vascular lesions occasioned by recent surgical advances, these aneurysms have been studied with respect to age, sex, race, size, type, site of occurrence, etiology, symptomatology, physical signs, duration of illness, and cause of death. These data reflect certain significant trends including the more frequent occurrence of aneurysms on an arteriosclerotic etiology over those on a syphilitic basis.

CLINICAL SIGNIFICANCE OF THE ASCHOFF BODY BASED ON MORPHOLOGIC AND HISTOCHEMICAL OBSERVATIONS. C. G. Tedeschi and (by invitation) B. M. Wagner and K. C. Pani, Hahnemann Medical College and Hospital, Philadelphia, Pa.

These observations are based on the analysis of 400 biopsies from left auricular appendages removed during the course of cardiac surgery. In all cases the preoperative diagnosis was "mitral valvular disease" of rheumatic origin. Extensive clinical laboratory evaluation established these cases as inactive. The pathologic findings led to a classification of the material into three main categories: (a) *Non-specific carditis, chronic type,* mainly characterized by fibrosis, scarring, and hyalinization

* Deceased.

of the collagen either of the endocardium or of the myocardium or both, in the absence of Aschoff nodules; (b) *Healed or healing rheumatic carditis,* including cases displaying senescent Aschoff nodules and often associated endocardial or myocardial fibrosis, scarring, or collagen hyalinization; (c) *Chronic, recurrent rheumatic carditis,* when an exudative type of inflammatory reaction was detectable in the nodule, in the endocardium or in the myocardium (independently from mural thrombosis); the structure of the characteristic cells of the nodule was well made out; the collagen fibers and ground substance were altered; and the myofibers showed marked degenerative changes. Since these unequivocal signs of an acute process were in every instance accompanied by alterations (fibrosis, scarring, collagen hyalinization) indicating a chronic lesion, this combination of recent and old changes was interpreted as representing an exacerbation of chronic rheumatic heart disease. Of the 400 cases examined, 325 (81.3 per cent) fell under the first category, 67 (16.8 per cent) under the second category, and 8 (2 per cent) under the third category.

The medical records of the individual patients in each of the three categories was analyzed for any clinical-laboratory manifestation of activity. Except for elevation of the erythrocyte sedimentation rate, which correlated with the pathologic classification, no clinical or laboratory indication of preoperative activity was found in any of the 400 cases, including the 8 which had displayed unequivocal evidence of acute carditis in the biopsy specimen. The postoperative course also failed to reveal in any of the cases signs, symptoms, or laboratory data which might suggest recrudescence of the rheumatic process.

Auricular thrombosis was found in 60 cases and this occurrence could not be correlated with the type of lesion in the appendage.

The findings in the biopsy specimens compared closely with those in other portions of the heart in 22 patients who died shortly after surgery. In relation to the problem of the origin of the multinucleated cell of the nodule from non-myogenic mesenchymal cells or from myofibrils, evidence was found in the present series indicating that both histogenetic mechanisms can be operative, the resultant being a lesion with distinct characteristics. It is felt that the giant cell of non-myogenic mesenchymal origin is unique for the rheumatic process, in contrast to the one originating from myofibers which can be found in rheumatic carditis as well as in a variety of processes in which damage of myofibers occurs. Detailed histochemical studies have demonstrated that the fibrinoid material of the ground substance is composed of an acid mucopolysaccharide-protein complex. It is suggested that the fragmented collagen fibers contribute to this fibrinoid material.

BACTERIAL ENDOCARDITIS PRODUCED BY THE TUBERCLE BACILLUS. R. Patterson Russell (by invitation) and Morgan Berthrong, Glockner-Penrose Hospital, Colorado Springs, Colo.

Authentic bacterial endocarditis caused by the tubercle bacillus is extremely rare. Occasionally, large extracardiac caseous masses erode the wall of the cardiac chamber and present beneath the endocardium. Even more commonly, during massive hematogenous spread of tubercle bacilli, miliary tubercles may develop in the endocardium. Neither of these conditions should be considered as comparable to bacterial endocarditis produced by the usual bacteria, even though the lesions may contain innumerable tubercle bacilli. A case of true tubercle bacillus bacterial endocarditis is presented, which developed on the deformed mitral valve which had been the site of a "mitral sling" operation for mitral regurgitation 2 years previously. Evidence was found to suggest that tubercle bacilli may have lodged initially in vegetations of acute bacterial endocarditis which had been cured by penicillin chemotherapy. At necropsy, myriads of acid-fast bacteria, growing in cords and dense clumps, were found both superficially and deep in a fibrinous and hyaline vegetation attached to

the mitral valve. The vegetation was larger and much smoother than the usual bacterial endocarditis. No other bacteria were found. The apparent initial source of the tubercle bacilli was an encapsulated primary lesion in the lung, in which no recent sign of spread or vascular invasion could be found. A discussion of previous cases and of criteria for diagnosis of tuberculous bacterial endocarditis was presented.

PATHOLOGIC FINDINGS ASSOCIATED WITH UNEXPECTED DEATH IN INFANTS AND CHILDREN. James B. Arey, Temple University School of Medicine and St. Christopher's Hospital for Children, Philadelphia, Pa.

Unexpected deaths, i.e., deaths prior to arrival at the hospital, in the Out-Patient Department, or within 24 hours after admission to the hospital accounted for almost one third of the necropsies at St. Christopher's Hospital for Children in the period 1949–1953 inclusive. The peak incidence of such deaths occurred during the first 6 months of life. They were more frequent during the winter and spring months. All patients dying within 24 hours after admission to the hospital were included in this series, irrespective of the presence or absence of previous hospital admissions. As a result, in approximately one fourth of the patients the presence of a pre-existing disease, often of a serious nature, was recognized prior to death. In the remaining patients, however, death occurred unexpectedly, often after manifestations of a mild illness were present for a variable, but usually short, period of time. The present study is based on the findings at post-mortem examination in 75 of these infants and children.

Infections were the leading cause of death, accounting for 33 of the 75 deaths. Pneumococcic and B hemolytic streptococcic septicemia, bronchopneumonia, leptomeningitis with and without the Waterhouse-Friderichsen syndrome, laryngotracheobronchitis, enteritis, osteomyelitis, and myocarditis were some of the infectious processes encountered in the present series. Congenital malformations were responsible for 14 deaths. These included endocardial sclerosis, hydranencephaly associated with cytomegalic inclusion disease, and a variety of congenital cardiac malformations. Miscellaneous lesions, including poisoning, intestinal obstruction or perforation, fibrocystic disease of the pancreas, galactosemia, erythroblastosis fetalis, and pulmonary hyaline membranes were responsible for almost one fourth of the deaths. In 11 patients in whom necropsies were performed, no adequate cause of death could be demonstrated.

There was nothing in this study to substantiate a diagnosis of status thymicolymphaticus and no deaths were attributed to suffocation. It is concluded that in the majority of infants dying unexpectedly an adequate cause of death can be demonstrated by a complete post-mortem examination, including histologic, bacteriologic, and chemical studies.

RÔLE OF PULMONARY HYPERTENSION AND THROMBO-EMBOLISM IN THE PRODUCTION OF PULMONARY ARTERIOSCLEROSIS.* R. M. O'Neal (by invitation) and W. A. Thomas (by invitation), Washington University Medical School, St. Louis, Mo.

Necropsy records and lung tissue sections were studied from 59 cases of congenital heart disease with anomalies permitting shunting of blood from the systemic to the pulmonary circulation (left-to-right shunt), 31 cases with pulmonary stenosis and septal defect (mostly tetralogy of Fallot), and 39 controls with normal cardiovascular systems. The following conclusions were reached.

(1) Pulmonary arteriosclerotic lesions characterized by fibrous intimal thickening were found in approximately 50 per cent of cases of congenital heart disease

* This study was supported by Grant H-1820 from the National Heart Institute, Institutes of Health, Public Health Service.

whether a left-to-right shunt or pulmonary stenosis was present. The incidence was similar in both groups, increased with advancing age, and in both abnormal groups was *100 per cent* in age groups over 10 years.

(2) Since it is reasonable to assume that the average of the pulmonary arterial pressures in patients with left-to-right shunt was higher than in the patients with pulmonary stenosis, it is apparent that hypertension was not a primary factor in the production of pulmonary arteriosclerosis in these cases. However, the severity of arteriosclerotic lesions was significantly greater in cases with left-to-right shunt than in those with pulmonary stenosis, suggesting that increased pressure and blood flow in these vessels may be etiologic factors of accessory importance.

(3) Fibrous intimal lesions were associated with pulmonary arterial thrombi in 70 per cent of the cases regardless of the type of anomaly present, suggesting that thrombi may be a primary factor in the production of these arteriosclerotic lesions.

(4) Pulmonary arterial thrombi were of similar frequency in cases with left-to-right shunt (25 per cent) and in cases with pulmonary stenosis (38 per cent).

(5) Because potential sources for emboli were found in 12 cases, an embolic origin of the arterial thrombi can be postulated.

(6) The transformation of a fresh thrombus to an arteriosclerotic lesion could occur through organization of the thrombus and subsequent contraction. Lesions which irresistibly suggest the stages in this process could be easily found.

EMBOLIZATION OF CEREBRAL TISSUE TO LUNGS FOLLOWING SEVERE HEAD INJURY. James B. McMillan (by invitation), Ohio State University, Columbus, Ohio.

Three cases are reported in which microscopic emboli of cerebral tissue were found in pulmonary arterioles. All 3 patients died following severe head injury with skull fractures, gross lacerations of the brain, and traumatic intracerebral hemorrhage. Lung sections showed numerous emboli of brain tissue distending the pulmonary arterioles, and in one case the embolization was accompanied by infarcts of the lung. In all cases glial cells were well preserved, and neurons and vascular structures could be identified in several of the emboli. A total of 199 complete necropsies were performed during a 17-year period on patients dying with severe head injuries, giving an incidence of 1.5 per cent for this lesion. Although the literature stresses the great rarity of its occurrence, the present report would indicate that traumatic embolization of cerebral tissue is not too uncommon. Careful histologic examination of numerous sections of the lungs is necessary, and two of the present cases were discovered only after careful restudy of the necropsy material. The validity of this report is further substantiated by a series of animal experiments.

GRANULOMATOUS PNEUMONITIS CAUSED BY SHORT-FIBERED ASBESTOS DUST (CHRYSOTILE). Paul Gross and (by invitation) Marian L. Westrick and James M. McNerney, Industrial Hygiene Foundation, Mellon Institute, Pittsburgh, Pa.

By a single intratracheal injection in rats of slightly less than 10 mg. of chrysotile fibers substantially shorter than 3 μ, but also including a few longer fibers, we have produced nodular granulomatous lesions leading to pulmonary fibrosis. Our findings are confirmatory of the pathogenicity of short-fibered asbestos dust reported by E. J. King, Clegg, and Rae in rabbits.

EXPERIMENTAL ASPIRATION PNEUMONIA: INFLAMMATORY AND REPARATIVE CHANGES PRODUCED BY INTRATRACHEAL INJECTIONS OF AUTOLOGOUS GASTRIC JUICE AND HYDROCHLORIC ACID. T. J. Moran, Presbyterian and Woman's Hospitals, Pittsburgh, Pa.

The effects of intratracheal injection of homologous gastric juice, hydrochloric acid, pepsin, and steapsin in rabbits have been studied. The changes produced in-

clude acute pulmonary edema and acute hemorrhagic pneumonia, both often fatal, and a variety of inflammatory and reparative lesions occurring after the first week, some of which also caused death. Production of acute pulmonary edema caused death in 4 to 90 minutes. In the fatal instances of acute hemorrhagic pneumonia, death occurred in 2 to 4 days. Death in other animals occurred in 3 to 13 weeks.

The changes occurring in animals dying or killed in 1 to 13 weeks included abscess formation, mononuclear and interstitial pneumonia, granulomatous reactions, interstitial thickening and fibrosis, atypical bronchiolar regeneration, sometimes resembling tumor, and vasculitis. The combination of atypical bronchiolar regeneration and interstitial fibrosis often produced localized areas resembling "acute diffuse interstitial fibrosis." Hyaline membranes were found in several pneumonic reactions occurring 1 day to several weeks after injection. Aspiration of food occurred in many animals receiving injections of hydrochloric acid, suggesting that a primary respiratory disease may predispose to aspiration pneumonia even in patients not in a recumbent position. The findings reported suggest that a variety of unexplained inflammatory, reparative, and fibrotic lesions of the human lung may be caused by aspiration of foreign material, especially gastric contents.

A Strain of Metastatic Epithelial Cells Derived from Long-Term Tissue Culture of Human Bone Marrow. Lawrence Berman and (by invitation) Cyril S. Stulberg and Frank Ruddle, Wayne University College of Medicine, Detroit, Mich.

In developing methods for long-term cultivation of human bone marrow it was observed that cultures passed through three morphologic phases similar to those reported by others using classical plasma clot methods. First, there is a myeloid phase during which the marrow cells retain their identity; second, there is a phase in which cells attaching to glass become rounded, expanded, and larger; and third, there is a phase of development of fibroblastoid cells which can be propagated indefinitely.

In a culture of bone marrow from a patient with carcinoma of the lung, several plaques of cellular material became visible among the usual proliferations of fibroblastic cells on the 51st day of continuous culture. The plaques consisted of masses of tissue from which emerged sheets of polygonal cells differing from the fibroblastic cells characteristic of cultures of this age. Because of the possibility of developing a useful stable strain of human cells for the study of viruses, it was decided to attempt to isolate these cells which we believe are epithelial cells, presumably of metastatic origin from a carcinoma of the lung. By means of transfers and subcultures we have continued the bulk propagation of this strain of polygonal cells which characteristically grow out in sheets in the fashion of epithelium since their original isolation in November, 1954. These cells, which we have designated as the Detroit^{-6} strain, have morphologic features indistinguishable from those of a known strain of human epithelial cells derived from a carcinoma of the cervix, originally isolated by Gey and now known as the HeLa strain. Nevertheless, there are certain topographic differences in the behavior of growth of these two strains.

Metabolic activity, as indicated by rate of fall of pH values during cell growth, is greater in the Detroit^{-6} strain, although an inoculum of 700,000 cells in 8 ml. of medium per flask results in a three-fold or better increase of cells in 5 days with each type. Preliminary observations on the susceptibility of the Detroit^{-6} strain to a spectrum of viruses includes studies made with poliomyelitis, Coxsackie, and influenza viruses. These pathogens, inoculated in the same concentrations at the same time into parallel cultures of HeLa and Detroit^{-6} cells have produced almost identical results. With both cell types poliomyelitis and Coxsackie viruses produced severe cytopathogenic effects in 48 hours, while influenza virus appeared harmless. It is possible that further investigation with additional viruses will yield evidence of differing susceptibilities. If not, the similarity of susceptibilities may indicate a fundamental unity in the behavior of human malignant epithelial cells of different origins.

COMPARISON OF HISTOCHEMICAL PROPERTIES OF NORMAL AND NEOPLASTIC SQUAMOUS EPITHELIUM. Alvan G. Foraker, University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas.

Comparisons have been made between certain histochemical reaction patterns of 25 examples of squamous carcinoma (skin or mucosal primaries, or lymph node metastases) with non-neoplastic squamous epithelium, using the following techniques: succinic dehydrogenase, alkaline phosphatase, phosphamidase, protein bound sulfhydryl and disulfide groups, glycogen, and other periodic acid-Schiff positive substances. Cytoplasmic evidence of dehydrogenase activity was found in the neoplastic cells, less marked in "maturing" cells near regions of keratinization or necrosis. The squamous carcinoma cells evidenced moderate nuclear reactivity and virtually no cytoplasmic reactivity to the standard alkaline phosphatase technique. This reactivity was much less than that seen in adjacent fibrous tissue and in blood vessel walls. Conversely, evidence of phosphamidase activity was considerable in nuclei and cytoplasm of neoplastic cells but was not found in significant quantity elsewhere in the tissues. Protein bound sulfhydryl reaction occurred in the cytoplasm and nucleoli of the neoplastic cells. The reaction was heavier in regions of keratinization. Disulfide reaction was prominent in regions of keratinization. A faint reaction was found in areas of tumor necrosis. "Maturing" squamous carcinoma cells toward the centers of tumor masses showed moderate glycogen reaction. This was seen in primary skin carcinoma as well as in primary mucosal carcinoma. The histochemical reaction pattern of the carcinoma cells was essentially the same as that of the normal skin or squamous mucosa, and revealed no findings qualitatively peculiar to neoplasia.

DETECTION OF SOLUBLE CARCINOMA ANTIGEN BY USE OF THE SCHULTZ-DALE TEST. Jack George Makari (by invitation), University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas.

The detection of neoplastic antigens by immunologic techniques has been handicapped by at least two factors: (1) the difficulty in detecting antigens which incite the production of univalent and fixed antibodies; and (2), of separating normal tissue antigens from specific neoplastic antigens. The Schultz-Dale test has been so adapted as to overcome both of these difficulties. Virgin female guinea-pigs have been actively sensitized to antigens prepared from carcinomatous tissues by the help of Freund's adjuvants. They were then sacrificed and their uterine horns tested in a Schultz-Dale bath. Two kymographs were used simultaneously to record contractions from the sensitized horn and from an unsensitized control horn. Normal group "O" plasma was added to both the sensitized and control horns. This results usually in a contraction of the sensitized, but not the control horn, due to the common component between the cancer antigens and normal group "O" plasma. By repeated additions, the sensitized horn may be completely desensitized to normal antigens in group "O" plasma. Following that, sera or plasmas from patients with carcinoma, other tumors, other diseases, and from patients from the Cancer Detection Clinic were tested. After each addition, Tyrode's solution in the Schultz-Dale bath was changed a sufficient number of times to allow the relaxation of the uterine horn to its base line.

It was found that sera from cases with carcinoma resulted in a specific contraction of the sensitized but not the unsensitized uterine horn after complete desensitization to group "O" plasma. By eliminating large-sized particles obtained by centrifugation at 3,600 r.p.m. and using those obtained between 3,600 and 10,000 r.p.m., an increase in sensitivity and a decrease in non-specificity occurred. Further work is in progress to evaluate on a large scale the possibility of using this test as an early diagnostic aid in individual cases and in the screening of carcinoma in the general population.

EXPERIMENTAL CARCINOGENESIS OF THE SKIN IN MICE: DOSE-RESPONSE RELATIONSHIPS. L. C. McTurk (by invitation), R. E. Eckardt (by invitation), W. E. Smith, and N. S. Cooper (by invitation), New York University—Bellevue Medical Center, New York, N.Y.

In attempting to quantitate tissue response to carcinogens, a 0.3 per cent solution of methylcholanthrene in acetone was applied 1, 3, 6, 9, 12, 15, 20, 30, or 50 times to the skins of mice. The number of animals that developed tumors in the various groups increased to a maximum of 100 per cent in the group given 30 applications. In a further experiment, graded concentrations of methylcholanthrene were applied to groups of mice three times a week throughout their lives while other groups received applications of each level of concentration three times a week for a total of 30 applications only. The concentrations studied were: 0.3, 0.2, 0.1, 0.075, 0.05, 0.025, and 0.01 per cent. A good linear relationship between tumor response and concentration of carcinogen was found in the groups given 30 applications only. The groups painted throughout their lives did not show this relationship. The experiments show that a limited number of applications affords a more suitable technique for estimation of relative carcinogenic potencies.

INFLUENCE OF THE INTENSITY OF EXPOSURE UPON THE NUMBER OF PULMONARY ADENOMAS INITIATED BY A SINGLE IN VITRO EXPOSURE TO ULTRAVIOLET IRRADIATION. Stanfield Rogers (by invitation), Duke University School of Medicine, Durham, N.C.

In vitro methods have been developed in this laboratory through which the mechanisms of oncogenesis may be studied in the same general way as has proved fruitful in studies of bacterial genetics. A single exposure of fetal lung tissue to ultraviolet irradiation of 2537 Å has proved an effective means of initiating neoplastic change under these *in vitro* conditions. This report is concerned with the effects of varying the intensity of irradiation upon the response in terms of number of tumors. The tumors are manifest in implants of irradiated tissue carried by normal mice. Increasing the intensity of irradiation increases the number of tumors appearing. However, the increase in number of tumors per unit amount of surviving tissue is less than would have been expected from the increase in the amount of irradiation applied. A similar effect has been obtained in the production of mutation in Drosophila ova by varying intensities of ultraviolet irradiation of this same wave length (Muller, 1954).

The tumors appearing in the limited time allowed for manifestation are in the general class of pulmonary adenomas. The range of variation in tumor size and morphology will be reported. In addition it will be shown that the variation in size from tumor to tumor of this class is determined by inheritable mechanisms influencing the morphologic appearance but to a less degree.

CARCINOLYTIC ACTION OF ANTIBIOTICS: PUROMYCIN AND ACTINOMYCIN D. Sidney Farber, Children's Medical Center and Harvard Medical School, Boston, Mass.

Recent studies have led to a search for carcinolytic properties in antibiotics. Puromycin and one derivative, an aminonucleoside, have been found by Olesen and his colleagues to have strong carcinolytic action against transplanted mammary tumors in mice. Our studies have confirmed this and have demonstrated a carcinolytic action, too, on transplanted leukemia in the mouse. Experiments concerned with the action of Actinomycin D, isolated by Waksman, have shown marked effect on transplanted tumor of the mouse against S-91 melanoma in both myeloid and lymphoid leukemia and mammary adenocarcinoma.

Possible mechanisms of action of these antibiotics were discussed.

LS Tumor, a Reticulum Cell Sarcoma of C57BL/6 Mice. C. O. Hathaway (by invitation) and E. A. Dowling (by invitation), Medical College of Alabama and Birmingham Baptist Hospitals, Birmingham, Ala.

A reticulum cell sarcoma, designated as the LS tumor, arose spontaneously in C57BL/6 ♀ mouse 5638. Now in the third passage, subcutaneous transplantations of this tumor into the right groin of 71 C57BL/6 mice produced 100 per cent growths. Necropsy studies have confirmed the evidence of metastases to lymph nodes and liver. The peripheral blood picture of mice bearing this tumor is not abnormal. In the first passage (36 mice) 50 per cent mortality was effected in 101 days; in the second passage (15 mice), in 65 days. This tumor has not yet grown in BALB/c or C57BR/cd mice.

Transplantation of the Parakeet Pituitary Tumor. Hans G. Schlumberger, Ohio State University, Columbus, Ohio.

Over 100 cases of spontaneous pituitary tumor in the parakeet have been examined in this laboratory. Of these, 10 tumors that had invaded the orbit or nasopharynx were transplanted as follows: 6 into the brains of 35 parakeets and/or 107 1-day-old chicks; and 4 subcutaneously in the breasts of 39 parakeets. In only 2 parakeets was there growth of the intracerebral transplants; in chicks, the tumor never grew. Three primary tumors grew as subcutaneous implants in parakeets. Of these, one is now in the fourth passage, 20 months after removal of the primary tumor. The transplants grow slowly, requiring about 2 months to gain a diameter of 5 mm.; the most rapid growth was seen in one transplant in the third passage that was 1.5 cm. in diameter 119 days after inoculation. A total of 15 transplants have been examined histologically and found to resemble the primary tumors which, because of the absence of granules in the cells, were tentatively identified as chromophobe tumors. Birds with growing transplants become very obese and show an elevated blood sugar that has reached a level above 1400 mg. per cent (normal: 250 to 385 mg. per cent). Whether this diabetogenic factor can be attributed to secretion of ACTH by the tumor is under investigation. No definite changes in the endocrine glands of these birds have been identified.

Neurogenic Tumors in the Duck: an Experimental Study. R. H. Rigdon, University of Texas Medical Branch, Galveston, Texas.

A variety of tumors (hemangiomas, fibromas, papillomas, lipomas, and squamous cell carcinomas) have occurred in the skin following the local application of methylcholanthrene. Recently, neurogenic tumors of three types have been observed in the skin: (a) ganglioneuroma, (b) neurofibroma, and (c) pacinian corpuscle tumors. These three latter lesions are similar to corresponding tumors as observed in man. We are unable to explain fully the origin of these neurogenic tumors in the duck. It would seem that there is some potential tissue in the duck that has the ability to reproduce neurologic structures of this wide variety when affected by methylcholanthrene.

Increased Spontaneous Occurrence of Fibroadenomas of the Breast in the Albino Rat Influenced by a High Fat Diet.* Jerome Benson (by invitation), Maurice Lev, and C. G. Grand (by invitation), Mount Sinai Hospital, Miami Beach, Fla., and University of Miami School of Medicine, Coral Gables, Fla.

During the course of an experiment involving the study of aging and other factors related to the production of arteriosclerosis in the Sprague-Dawley albino rat, pen-

* Aided by a grant from the Leon Lowenstein Foundation, New York, N.Y.

bred animals of various ages were given the following diets: (1) Purina laboratory chow with 20 per cent olive oil, with and without 2 per cent cholesterol, (2) Purina chow with desiccated thyroid, with and without 2 per cent cholesterol, (3) Purina chow with thiouracil, with and without 2 per cent cholesterol, and (4) Purina chow with I^{131}, with and without 2 per cent cholesterol. Within all eight of the above experimental groups we observed the development of rapidly growing fibroadenomas of the breast in various areas along the mammary line in 11 per cent of 277 animals. This was in contrast to 3 per cent in the control group of 232 animals. In both the control and experimental groups, these tumors were found only in animals 18 months or older. In the control group, these tumors were limited to the female while in the experimental group a few males also demonstrated tumors. There was no appreciable difference between animals with or without cholesterol feeding. There was distinct delay in the development of tumors in animals getting thyroid, and suggestive evidence of accelerated onset in animals with I^{131}. The tumors were easily removed, tended to be multiple, and occasionally recurred at the site of operation or at an entirely different focus along the mammary line. In two instances, removal of a fibroadenoma was followed by the development of a sarcoma. Only the malignant tumors could be grown *in vitro*. The level of blood lipids in the experimental group, in general, was higher than that of the controls. It is believed that the high incidence of benign mammary tumors in the experimental group is related to hypernutrition in the form of a high fat diet.

PATHOGENESIS OF BOVINE OCULAR SQUAMOUS CELL CARCINOMA: HISTOPATHOLOGIC AND CLINICAL STUDIES. E. Staten Wynne (by invitation) and William O. Russell, University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas.

In a previous communication preliminary findings were presented on the pathologic anatomy of ocular squamous cell carcinoma of bovines. Phases noted were "plaques" of keratotic thickening, squamous papillomas, and varying types of squamous carcinoma. The present study was undertaken to secure more complete information on the various lesions and to evaluate the developmental relationship of the benign to the malignant phases of the disease. Lesions from over 800 animals were obtained from commercial slaughter and were sectioned for pathologic examination. Also, observations were made of more than 350 affected animals under field conditions over periods up to 2 years. The benign precursor lesions may be divided on the basis of site of origin into: lesions of the conjunctival sac (including limbus, cornea proper, membrana nictitans, caruncle, bulbar conjunctiva, and lid conjunctiva), and lesions of the skin of the lid and modified skin of the lacrimal lake.

Conjunctival lesions are best illustrated by those at the limbus. The earliest change, designated as a "plaque," appears grossly as a pearly grayish white area of thickened epithelium. Microscopically, there is epidermidalization or squamous metaplasia with dyskeratosis which may be leukoplakic or of a Bowen type. As in analogous human lesions, the leukoplakic type of dyskeratosis shows hyperkeratosis and a marked inflammatory reaction. The hyperplasia may involve any or all layers of the epithelium. The Bowen type of dyskeratosis ordinarily does not exhibit hyperkeratosis and has minimal inflammatory reaction. Advanced plaques of either type form well defined rete pegs, with the intervening fibrous stroma becoming convoluted. As development continues, plaques may form papillomas with fronds consisting of fibrous tissue covered by epithelium.

Early hyperplastic lesions of skin of lid are (1) keratomas and (2) what we have termed "boggy" lids. The former consist of horn-like outgrowths of hyperkeratotic epithelium basically similar to plaques, and, in fact, representing cutaneous horns. The "boggy" lid is a generalized acanthotic change with minimal keratosis and a

marked subepithelial inflammatory reaction. Papillomas of lid are rare. Any of the benign lesions mentioned may undergo malignant change to form squamous cell carcinoma of varying degrees of differentiation. The stage of carcinoma *in situ* has been observed in plaques, keratomas, and papillomas.

PIGMENT PRODUCTION AND BIOLOGIC BEHAVIOR IN 380 CASES OF MALIGNANT MELANOMA. William O. Russell and (by invitation) Samira Guraieb, William W. Wiltberger, and R. Lee Clark, Jr., University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas.

The production of demonstrable melanin in malignant melanoma is of variable occurrence and undetermined significance. Recent investigations indicating a high tyrosinase activity in malignant melanocytes have raised questions as to a possible relationship between the visible pigment produced by the cells and their rate of growth. The degree of pigment formation determined from sections stained with hematoxylin and eosin has been reviewed in 380 cases of microscopically proved malignant melanoma. Tumors have been classified as melanotic if pigment formation occurred in any part and as amelanotic in the absence of pigment. Of the study group, 237 (62 per cent) were melanotic, and 143 (38 per cent) were amelanotic. Pigment in the melanotic group was graded from 1 to 4. There were 49 graded I, 27 graded II, 28 graded III, and 34 graded IV.

The criterion for evaluation of biologic behavior was the length of survival after the initial symptom of disease, wherever verified in sequence by pathologic diagnosis. Those cases in which death occurred from metastases within 1 year after the initial symptom were classified grade IV; within 1 to 3 years, grade III; and within 3 to 5 years, grade II. Grade I was reserved for patients surviving past the 5-year period before dying of their disease, or surviving past the 5-year period for an indefinite interval thereafter. There were 205 cases sufficiently complete for this evaluation. There were 58 graded I, 29 graded II, 70 graded III, and 48 graded IV. Since the cases in this series were collected over an 11-year period, with over 50 per cent being seen within the past 3 years, the opportunity for clinical evaluation is necessarily limited in its scope as compared to the opportunity for evaluation of pigment formation. Pigment production will be correlated with biologic behavior, age, sex, color of skin, and anatomical site.

SYMPOSIUM ON NEW APPROACHES TO THE STUDY OF RENAL DISORDERS

Referee (by invitation of the Council): Jean Oliver

REDUCTION OF POST-NEPHRECTOMY HYPERTENSION BY HOMOGENOUS RENAL TRANSPLANT MEDIATED BY MEANS OTHER THAN DESALTING AND DEHYDRATION. E. E. Muirhead and (by invitation) J. A. Stirman and W. Lesch, University of Texas Southwestern Medical School, Dallas, Texas.

Eleven dogs were subjected to bilateral nephrectomy and maintained by peritoneal irrigation two or three times daily. A positive fluid balance accrued, amounting in 8 to 12 days to 1.7 to 3 liters in 5 dogs and 5 to 10.5 liters in 6. All animals became hypertensive. The increment in mean blood pressure varied between +25 and +60 mm. of Hg (av. 45 ± 12). During this interval 4 animals displayed no weight change, 4 gained 0.8 to 2.2 kg., and 3 lost 0.6 to 2 kg.

On the 8th to 15th day a kidney from a normal dog was transplanted to the neck of the hypertensive nephrectomized dog, the anastomosis being established between the renal and carotid arteries and the renal and jugular veins. The donor kidney was

ischemic for 20 to 40 minutes. Urine flowed in 5 minutes and persisted usually for 7 to 14 days. The blood pressure receded within 24 hours and remained depressed for an average of 5.5 days, following which it tended to become labile and returned to hypertensive levels in 8 cases. The mean values indicated a change from +45 to +20 to 28 mm. of Hg. The 5 examples yielding the greatest change were as follows: +70 to +10 to 30; +50 to +10; +45 to +15; +55 to +15 to 25, and +60 to +20. Peritoneal irrigation was continued for 5 to 10 days after the operation, making water and salt available for excretion. While the blood pressure was lowered, the weight remained unchanged in 7 dogs, was slightly lowered but still above the control in 2, and was lowered 1 to 2 kg. in 2. Throughout, the animals received a diet containing 35 to 65 cals./kg./day derived from protein, fat, and carbohydrate. Three dogs treated in a like manner and subjected to a sham operation failed to show depression of the blood pressure. The observations are considered consistent with the view that the transplanted kidney lowers the blood pressure of hypertensive nephrectomized dogs by mechanisms other than the loss of salt and water.

PROLONGED FUNCTIONAL SURVIVAL OF A RENAL HOMOTRANSPLANT IN MAN: MORPHOLOGIC OBSERVATIONS WITH CLINICAL CORRELATION. G. J. Dammin and (by invitation) D. M. Hume, J. P. Merrill, B. F. Miller, and G. W. Thorn, Peter Bent Brigham Hospital and Harvard Medical School, Boston, Mass.

Functional survival for almost 6 months of a renal homotransplant in man has not been observed heretofore. The recipient (G. W.) was a 26-year-old physician with chronic glomerulonephritis; the donor, a 37-year-old female patient with rheumatic heart disease who died during a cardiac operation. The renal homotransplant was placed into the thigh and femoral anastomoses and a cutaneous ureteral orifice established. During the period of function, the output of urine was 1500 to 2000 ml. per day, blood urea nitrogen fell from 244 to 33 mg. per cent, and clinical improvement was apparent. Hypertension continued, congestive failure and uremia with reduced renal homotransplant function appeared during the last week, and the patient expired 6 months postoperatively.

The clinical diagnoses were confirmed at post-mortem examination. The kidneys together weighed 135 gm.; the heart weighed 380 gm., and showed fibrinous pericarditis. The renal homotransplant weighed 340 gm., approximately twice the weight of the other kidney of the donor. No infection was observed at the operative site or in the kidney. Major branches of the renal artery showed advanced atherosclerosis. Interstitial edema obscured the architecture as observed on the cut surface. Microscopically, in addition to chronic glomerulonephritis, the patient's own kidneys showed the tubular alterations of recent ischemic nephrosis. Cholesterol, phospholipids, and free fat were demonstrated histochemically in the tubules. There was moderate atherosclerosis of the aorta and of the renal and coronary arteries.

In the homotransplant, the glomeruli were essentially normal and resembled those of the donor's other kidney. There was advanced focal atrophy of tubular portions of the nephrons, apparently occurring independently, since no glomerular involvement was observed. In other areas the tubules were normal or hypertrophied. None contained lipids. No tubulorrhexis or other manifestations of ischemic nephrosis were noted. There were foci with lymphocytes and plasma cells, many of which showed cytoplasmic pyroninophilia. Cholesterol, phospholipid, and free fat were found in the thickened intima of the major branches of the renal artery. There was complete endothelization of the vascular anastomoses. The recipient epidermis had grown over and into the ureter, which showed intact smooth muscle and vascular supply. These observations suggest a high order of donor and recipient compatibility. The outcome is attributed more to chronic glomerulonephritis with hypertension, uremia, and congestive failure and to ischemic nephrosis, than to intrinsic failure of the renal homotransplant.

EFFECTS OF PROLONGED EXPERIMENTAL HYPERTENSION IN THE DOG WITH A STUDY OF PERIODIC RENAL BIOPSIES OVER A SEVEN-YEAR PERIOD. G. J. Dammin and (by invitation) M. L. Goldman, H. A. Schroeder, and M. G. Pace, Peter Bent Brigham Hospital and Harvard Medical School, Boston, Mass.; and Barnes Hospital and Washington University School of Medicine, St. Louis, Mo.

Hypertension was produced in dogs by either bilateral moderator nerve resection (group I), unilateral application of a Goldblatt clamp or silk pouch (group II), or a combination of these procedures (group III). Thirty of 56 dogs developed hypertension and of these, 3 in group I, 5 in group II, and 3 in group III were observed for a 7-year period. A wide range of ages was represented, from less than 1 year to advanced age. In a report on observations made on the renal biopsies during the first 4 years, it was noted that the most pronounced change in the glomerulus consisted of a uniform thickening of the capsular basement membrane. Less prominent were a thickening of the basement membrane of the glomerular tuft and an increase in Schiff-positive intercapillary material in the tuft. Most of the dogs showed these alterations which appeared during the first year of hypertension and in all three of the experimental groups. No significant alterations were noted in the arterioles at that time.

At the end of the 7-year period, at which time post-mortem examinations were made, the changes most apparent still consisted of a marked increase in thickness of the basement membrane of the glomerular tuft and an increase in Schiff-positive intercapillary substance. An increase in cells, probably endothelial in origin, accompanied the increase in Schiff-positive intercapillary substance which was usually concentrated at the hilum. Changes somewhat comparable to these were noted in aged dogs. However, in the experimental dogs, regardless of age, they occurred during the first year of hypertension. An alteration not observed at the end of the 4-year period was a thickening of the arterioles adjacent to the glomerular hilum. Of the 10 dogs observed throughout the 7-year period, 5 had moderate, 4 had slight, and one had no arteriolar thickening. Hypertrophy and slight hyperplasia of the media appeared to account for the thickening of the arteriolar wall. Further studies will attempt to determine whether alteration of the intima and adventitia also contribute to the arteriolar thickening. No comparable arteriolar thickening was encountered in aged dogs used as controls.

Hypertension produced in the dogs as described, has resulted, in the course of 7 years, in the following renal alterations listed in order of decreasing prominence and frequency: (1) marked thickening of the capsular basement membrane, (2) increase in Schiff-positive intercapillary substance, (3) moderate thickening of the basement membrane of the glomerular tuft, and (4) increase in arteriolar wall thickness.

FINE STRUCTURE OF THE RENAL GLOMERULUS. HISTOCHEMICAL AND ELECTRON MICROSCOPIC OBSERVATIONS. James F. Rinehart and (by invitation) Marilyn Gist Farquhar and Eleanor Gould, University of California School of Medicine, San Francisco, Calif.

This report is concerned with the fine structure of the renal glomerulus in normal and abnormal states as revealed by electron microscopy and appropriate histochemical staining methods.

As seen with the electron microscope the glomerulus shows an elaborate organization and an exquisitely delicate, fine structure. A continuous layer of fragile, lace-like, endothelial cytoplasm lines the inner aspect of the basement membrane. The basement membrane proper appears to be a differentiated product of endothelial cytoplasm. It is of relatively uniform density and of the order of 0.08 μ in thickness. Under certain stresses it appears to show a very fine porosity. Based upon histochemical studies, it is believed to be in the nature of a glycoprotein gel. Electron

microscopic studies have, for the first time, revealed the remarkable organization of the epithelium which can be adequately delineated only by illustration. Innumerable extensions of epithelial cytoplasm—foot processes—are regularly inserted on the outer aspect of the basement membrane. Based on staining properties, it is believed that the epithelial cytoplasm is of a mucinous character.

From studies of the fine structure of the glomerulus under various stresses certain physiologic mechanisms involved in glomerular "filtration" are suggested. It appears that plasma may traverse the endothelial cytoplasm via cytoplasmic vesicles. The basement membrane proper would appear to serve as a fine filter allowing free transfer of solutes but preventing escape of large molecules such as the blood proteins. It is suggested that the elaborately organized glomerular epithelium may play an active rôle in elaboration of the glomerular filtrate. Examples of certain alterations seen in glomerular disease were described and illustrated.

HISTOCHEMICAL LOCALIZATION OF ENZYMATIC ACTIVITIES IN VARIOUS PORTIONS OF THE MAMMALIAN NEPHRON. M. Wachstein and (by invitation) E. Meisel, St. Catherine's Hospital, Brooklyn, N.Y.

Staining techniques for the demonstration of various enzymes were applied to kidneys from rat, mouse, rabbit, dog, cat, and human (surgically removed kidneys and necropsy material). Unfixed frozen sections were used throughout in order to minimize loss of enzymatic activity. Certain variations in the distribution pattern of these enzymes occurred in the various species examined. Non-specific alkaline phosphatase, 5-nucleotidase, and glucose-6-phosphatase were localized prominently in the proximal convoluted tubules with variations in the staining intensity of the terminal portions in various species. With 5-nucleotidase (lead technique, pH 7.2) in addition, the glomeruli of mouse and human stained and there was found activity in capillaries, most pronounced in the human kidneys. Acid phosphatase, although most active in the proximal convoluted tubules, was found also in other tubular segments including the collecting tubules. In the human kidney, in addition, thin limbs of Henle and glomeruli showed positive staining reaction.

In view of recent evidence that a pH of 7.2 is optimal for non-specific phosphatase activity in the living cell, a modified lead technique at this pH was used. With this method a general reaction in the cytoplasm of the tubules of most portions of the nephron, including the collecting tubules, was observed. In man, the thin limbs of Henle's loop were also active. Esterase and glucoronidase activity were found in all tubular elements in the cortex and there was also some staining in ascending limbs of Henle's loop and collecting tubules.

When stained for oxidative enzymes the distribution patterns were quite similar in all species examined. Endogenous dehydrogenase and succinic dehydrogenase were distributed in a similar fashion, with marked activity in most tubular segments with the exception of thin limbs of Henle and the distal portions of the collecting tubules. Maximal staining was seen in ascending limbs and distal convoluted tubules. Diphosphopyridine (DPN) diaphorase was strongly active in the proximal convoluted tubules but there was considerably less activity in the ascending limbs and distal convoluted tubules. Thin limbs of Henle and glomeruli gave a distinct staining reaction, particularly strong in human kidneys. In general, the staining intensity for triphosphopyridine (TPN) diaphorase was less intense. The distribution pattern resembled that for diphosphopyridine (DPN) diaphorase. The presence of a number of enzymes within thin limbs of Henle's loop and collecting tubules would indicate a more active rôle of these segments for the functional activities of the kidneys than is usually assumed. Under experimental conditions (acute necrosis, regeneration, hydronephrosis), significant changes in the enzymatic staining patterns were

observed. In human necropsy material surprisingly good results were obtained with some of these techniques, particularly those for the various dehydrogenases.

ISCHEMIC GLOMERULI AND THEIR SIGNIFICANCE FOR GLOMERULAR STRUCTURE. J. F. A. McManus, University of Alabama Medical Center, Birmingham, Ala.

Twenty-five years ago, MacGregor identified the changes in the renal glomerulus in essential hypertension. They were described as consisting "of a decrease in size and simplification of the glomerulus with a marked thickening and wrinkling of the glomerular basement membrane." The correctness of this pattern of glomerular change in arteriosclerosis has been confirmed by Allen, McManus, and, most recently, by Jones. In addition to identifiable arteriosclerotic or ischemic changes, pathognomonic glomerular lesions have been described by me in the major renal diseases and in many diseases primarily non-renal in origin. Increasing clinical use of percutaneous renal biopsy makes the precise identification of glomerular lesions more important.

There is by now good agreement that there is an intercapillary space. Singleness of the glomerular basement membrane by light microscopy and the absence of a second cell type within the basement membrane, different from the endothelial cells, have led a number of workers to doubt the existence of a mesangium, or axial space, or intercapillary space. These difficulties appear to have been resolved by the recent description of the anatomy of the glomerulus by Mueller, Mason, and Stout. To quote: "The cell bodies of the glomerular endothelium are syncytial and form a stalk around which the capillary rete courses in and out of the glomerulus. This endothelial stalk constitutes what has been called the intercapillary space, the glomerular stalk, or mesangium. It is composed of endothelial cells whose adjacent boundaries cannot be defined."

The explanation of certain features of the ischemic glomerulus becomes possible upon the basis of the structure described by Mueller, Mason, and Stout. There are further evidences, in turn, for the intercapillary space in the sparing of ischemic glomeruli in intercapillary glomerulosclerosis and acute glomerulonephritis. The frequently repeated suggestion that intercapillary glomerulosclerosis is related directly to arteriosclerosis or even "is the logical end stage of primary, slowly progressive arteriosclerosis of the glomerulus" is easily controverted by these data. Studies of ischemic glomeruli with the PAS procedure or Ritter and Oleson stain suggest that the "simplification" of the glomerulus is due to opening of the intercapillary space and presumably due to damage to the endothelial and/or mesangial cells which normally occupy it. The absence of an intercapillary space in ischemic glomeruli explains the lack of the hyalin of intercapillary glomerulosclerosis in such glomeruli.

IMPROVED METHOD FOR THE HISTOCHEMICAL LOCALIZATION OF OXIDATIVE ENZYMES IN THE KIDNEY WITH TETRAZOLIUM. Emmanuel Farber, Tulane University School of Medicine, New Orleans, La.

During a study of the mechanism of staining for certain oxidative enzymes in the kidney with tetrazolium salts, in was observed that unreduced blue tetrazolium (BT) was bound non-specifically to the cytoplasm of all cells. In sections previously incubated with BT without substrate, the bound BT could subsequently be selectively reduced in cells containing enzyme by the addition of appropriate substrates and co-factors. These unpublished findings have recently been confirmed by Wattenberg for neotetrazolium. This and other evidence suggested that, during the usual staining procedure, the enzymes were interacting with bound unreduced BT rather than with BT dissolved in the medium. BT reacts relatively slowly with the succinic dehydrogenase system and with diphosphopyridine nucleotide (DPN) diaphorase and triphosphopyridine nucleotide (TPN) diaphorase. Many oxidation-reduction indicators, such as methylene blue, are also bound by cells and react

more rapidly with many enzymes. However, they are not satisfactory histochemical stains. Some of these dyes, when reduced, are in turn capable of reducing BT. Therefore, the possibility was tested that addition of these more reactive dyes to the medium might accelerate the rate of deposition of BT. Frozen sections of kidney and other organs were incubated in the appropriate medium for each of the three enzymes mentioned (Seligman *et al.*, Farber *et al.*). In some flasks a small amount of one of several indicators was provided in addition to BT. Staining was frequently more rapid, more intense, and more reproducible than with BT alone. Different indicators were found to be best for each of the three enzymes studied. For the succinic dehydrogenase system, methylene blue (50 μg.) gave the best results. For DPN diaphorase, thionine (50 μg.) was the dye of choice. For TPN diaphorase, a mixture of thionine (50 μg.) with Nile blue A (50 μg.) was found satisfactory. With BT supplemented by these dyes, excellent staining for each of the enzymatic activities was reproducibly obtained with incubation periods of 5 to 10 minutes as compared to 30 to 90 minutes ordinarily required with the original methods. The staining patterns were the same as with BT alone. It is probable that these soluble dyes are non-specifically bound to the cytoplasm along with unreduced BT and that they act catalytically by accelerating the transfer of H^+ and electrons from the enzymes to blue tetrazolium at sites of enzyme activity. This method makes it unnecessary to incubate tissues for long periods of time and makes it possible to stain tissues with lower enzyme activity than could be stained by the original methods.

HISTOCHEMICAL LOCALIZATION OF CERTAIN OXIDATIVE ENZYMES DURING THE PRODUCTION OF INFARCTS OF THE RAT KIDNEY. Evelyn Richardson Peters (by invitation) and Emmanuel Farber, Tulane University School of Medicine, New Orleans, La.

The alterations occurring in a tissue during the interval between occlusion of a vessel and the production of a characteristic infarct have been studied primarily by histologic methods. In an effort to learn more about the metabolic changes preceding cell death, the histochemical localization of the succinic dehydrogenase system (Seligman *et al.*), diphosphopyridine nucleotide (DPN) diaphorase, and triphosphopyridine nucleotide (TPN) diaphorase (Farber *et al.*), has been studied in the rat kidney during the production and healing of infarcts. Infarcts were produced by passing a ligature beneath a branch of the renal artery and bringing the ends out through the muscle and skin. The ligature was pulled tight and tied on the external skin surface, thus occluding the artery. Release of the occlusion could be accomplished, without damaging the vessel, by cutting the ligature on the skin surface and pulling it free. Temporary occlusion of a branch of the renal artery for periods of from 30 minutes to 3 hours resulted in the production of a typical infarct within 2 to 3 days.

Staining for succinic dehydrogenase, DPN diaphorase and TPN diaphorase of a kidney immediately after a branch of the renal artery had been ligated for 30 minutes revealed no deviation from the usual staining pattern in the region supplied by the branch. Similar studies 4 to 5 hours after a 30-minute occlusion showed a uniform decrease in enzyme staining in the affected region. Occlusion for periods of 2 hours resulted in zonal differences in enzyme staining. In the case of succinic dehydrogenase and of DPN diaphorase, decreased staining occurred in the inner cortical and medullary zones of the kidney. With the TPN diaphorase system no staining occurred in any zone. After a 3-hour period of occlusion no staining was observed in the affected portion of the kidney with any of the three enzyme systems studied. Duplicate sections revealed hyperemia of the affected portion of the kidneys without evident necrosis when stained with hematoxylin and eosin.

Preliminary studies were made on healing infarcts, produced by temporary occlusion of a branch of the renal artery for 30 minutes. Enzyme studies of the kidney 4 to 6 days later showed that the TPN diaphorase system was the earliest to recover. Hematoxylin and eosin sections of the same kidneys show marked regeneration of all portions of the renal tubules in the infarcted area. These studies are being extended to include animals followed for longer periods after temporary occlusion of a branch of the renal artery.

NEPHROTIC SYNDROME INDUCED IN RATS BY AMINONUCLEOSIDE.* John M. Craig, Children's Medical Center and Harvard Medical School, Boston, Mass.

Daily parenteral injection of 10 to 100 mg. per kg. of 6-a-methylamino purine, 3 amino d-ribose, an aminonucleoside related to "puromycin"† in rats results in edema, ascites, proteinuria, hyperlipemia, hypoproteinuria, and azotemia. The clinical symptoms are related to alterations in the basement membrane of the glomeruli and in the phosphatase activity, the mitochondrial pattern and lipid distribution in the renal tubules. Over short periods of administration these lesions are reversible with reconstruction of the glomerular and tubular pattern and relief of the clinical symptoms. The progress in changes in the renal tubules and their repair at various dosage levels will be discussed.

GLOMERULAR LESIONS AND THE NEPHROTIC SYNDROME IN RABBITS GIVEN SACCHARATED IRON OXIDE INTRAVENOUSLY. John T. Ellis, New York Hospital—Cornell Medical Center, New York, N.Y.

Renal lesions involving principally the glomeruli and functional alterations identical with those of the nephrotic syndrome have been observed in rabbits following the injection of massive amounts of saccharated iron oxide (S.I.O.). The rate of protein excretion in the urine, and the concentration of the serum protein fractions, cholesterol, and non-protein nitrogen were studied serially over prolonged intervals of time in animals given single or repeated injections of S.I.O., and these observations were correlated with structural changes found in the kidneys after biopsy or necropsy.

Eight of 15 young rabbits given a single injection of a large amount of S.I.O. developed proteinuria, hypo-albuminemia, hypoproteinemia with elevated alpha globulin, and hypercholesterolemia. In a typical experiment, proteinuria began abruptly 5 days after the injection and 3 days later reached a maximum of 1800 mg. per 24 hours; it continued at over 800 mg. per 24 hours for 18 days, thereafter diminishing gradually. During this period of severe proteinuria, serum albumin fell from 5 gm. per cent to 0.59 gm. per cent and conversely the serum alpha globulin increased from 0.73 to 1.16 gm. per cent and the serum cholesterol rose from 90 to 250 mg. per cent. Each of 10 rabbits given multiple injections of smaller amounts of S.I.O. over periods up to 1 year developed similar changes of longer duration than those observed in rabbits given a single massive injection of S.I.O. In addition, subcutaneous edema was conspicuous in 5 of the 10 rabbits. There was no azotemia or hematuria.

Brown granular precipitates, often forming casts of the capillaries, were found regularly in the kidney, liver, spleen, and other viscera of 20 rabbits given a single massive injection of S.I.O. and sacrificed after intervals of 15 minutes to 7 days. These precipitates were comprised, at least in part, of iron as shown by special

* Aided by grant #C1975c of the National Cancer Institute, National Institutes of Health, Bethesda, Md.

† Kindly supplied by Lederle Laboratories, the American Cyanamide Co., Pearl River, N.Y.

stains. In the rabbits studied over prolonged intervals, the renal lesions affected the glomeruli principally, the changes in general paralleling the proteinuria. The earliest recognizable glomerular alterations consisted of marked dilatation of Bowman's space with protein precipitate, together with pyknosis of a few endothelial cells and margination of leukocytes and endothelial siderosis. Later alterations were glomerular siderosis, proliferation of the glomerular and capsular epithelial cells, focal degeneration of the glomerular basement membrane, and varying degrees of glomerular fibrosis. Although the exact mechanism by which the initial glomerular injury is produced remains unknown, the findings make it seem likely that the damage may have resulted from anoxia of the glomerular capillaries produced by the intravascular precipitates.

NEW APPROACHES TO THE STUDY OF RENAL DISORDERS. Jean Oliver (referee*), Renal Research Unit, Overlook Hospital, Summit, N.J.

PATHOGENESIS OF EXPERIMENTAL GLOMERULONEPHRITIS: HISTOPATHOLOGIC DEMONSTRATION OF GLOMERULAR-LOCALIZING ANTIBODIES.† Robert C. Mellors, Memorial Center, New York, N.Y.

The rôle of antigen-antibody reactions in the pathogenesis of certain types of glomerulonephritis, as well as of a variety of other diseases thought to be associated with allergy and hypersensitivity, has been discussed widely during the last century. Clinical observations, serologic findings, morphologic studies, and investigative work with experimental animals lend indirect support to immuno-allergic pathogenesis. The unique feature of the present study is the use of a recently described microfluorescence method for demonstrating the histologic sites of localization of antibodies *in vivo*. It is found that antibodies are localized in the glomeruli of the kidneys of rabbits having glomerulonephritis of the acute proliferative, the exudative, or the crescentic type induced by the injection of foreign protein (bovine gamma globulins). Such localization is clearly an essential requisite for the allergic pathogenesis of glomerulonephritis.

LOCAL RENAL SHWARTZMAN PHENOMENON. B. Black-Schaffer and (by invitation) Uriel Garcia-Caceres, University of Cincinnati College of Medicine, Cincinnati, Ohio.

Bilateral renal cortical necrosis, in the rabbit, has been called the characteristic lesion of the generalized Shwartzman phenomenon. Since numerous bacterial toxins without significant Shwartzman potency, as well as severe shock alone, may produce the same renal lesion, it is thought that the renal lesion in the generalized Shwartzman phenomenon is a non-specific reaction to severe shock. The left renal arteries of a series of rabbits were inoculated directly with Shwartzman toxin. Twenty-four hours later the same toxin was inoculated intravenously with the production of a hemorrhagic lesion in the same kidney. The ability of the kidney to react to the Shwartzman toxin may be significant since allergic reactions which are amplified by means of the Shwartzman phenomenon are believed to play an important rôle in glomerulonephritis.

RENAL SHWARTZMAN PHENOMENON IN TRICHINOSIS. Raymond Yesner, Veterans Administration Hospital, West Haven, Conn.

Experimentally, one or more intravenous injections of bacterial filtrate give rise to multiple foci of thrombosis without endothelial reaction, particularly in the

* By special invitation of the Council.

† This article will appear in a subsequent issue of *The American Journal of Pathology*.

lungs, spleen, and liver—all organs of slow circulation. This is interpreted to be a direct toxic effect on the blood components. It is noteworthy that the kidney is spared. In contrast, two intravenous injections of bacterial filtrate, optimally 24 hours apart, produce arteriolar thrombosis in the kidney with cortical necrosis. This is interpreted to be in the nature of a Shwartzman reaction. In man, the Shwartzman reaction in the kidney has been observed in bacterial, viral, and protozoan infestations. To this is now added a case of helminthic infestation, trichinosis. Sensitivity phenomena were manifested by eosinophilia and arthralgia. Hematuria and anuria preceded death. The causative organisms were found in large numbers, encysting and encysted. Homogeneous eosinophilic thrombi occupied the afferent arterioles and glomerular capillaries of the kidneys with concomitant cortical necrosis. This phenomenon is identical with the Shwartzman reaction produced experimentally by two intravenous injections of bacterial filtrate.

PATHOGENETIC STUDIES IN THE EXPERIMENTAL HEPATORENAL SYNDROME USING MICRO-ANGIOGRAPHY IN THE AGONAL PHASE. C. Neville Crowson (by invitation), Robert H. More, and John B. King (by invitation), Queen's University, Kingston, Ont., and Edinburgh University, Edinburgh, Scotland.

We have noted a significant correlation between various forms of liver damage and a nonspecific degenerative lesion of glomeruli and tubules of the kidney (glomerulotubular nephrosis) in human necropsy material, temporary acute surgical hepatic ischemia in the rabbit, and toxic (CCl_4) liver damage in the rat. In addition, we have shown that the vasospastic necrotizing activity of pituitrin on renal tubules is potentiated in the presence of CCl_4 liver damage and this effect is probably not due to simple adjuvant action of CCl_4 and pituitrin on the renal tubular epithelium. This suggests a humoral homeostatic rôle of the liver in renal hemodynamics.

It seemed that renal ischemia might be the cause of the tubular changes. To study this proposal a blood-replacement micro-arteriographic technique for use during the agonal phase is being investigated. The use of this method implies the assumption that small vessel alterations will continue during the agonal phase and be demonstrable in fixed injected tissues. The technique involves canulation of the descending thoracic aorta under deep ether anesthesia, following intracardiac heparin. The vascular bed is perfused with heparinized N-saline for 20 seconds and the injection medium is then run in at a pressure of 120 mm. of Hg. Various media have been tried including heavy metal insoluble salts for microradio-arteriography and a phthalocyanine derivative (Monastral Fast Blue) for microphoto-arteriography. A 20 per cent suspension of bismuth oxychloride in 10 per cent plasma protein proved fairly satisfactory for radiologic studies. With this material, there appeared to develop an obstruction of the fine vascular beds of the liver and kidney in both CCl_4 and $HgCl_2$ poisoning. However, heavy metal salts of this order agglutinate at variable rates on standing, tending to produce artifactitious filling defects. For this reason, Monastral Fast Blue, a radiolucent material of very fine particle size, is being tested at the present time, employing photomicrographs of thick cleared sections of injected tissues.

Replacement micro-arteriography during the agonal phase offers many advantages over *in vivo* blood-dilution arteriography and renal function techniques because it presents a more accurate demonstration of the site of vascular alteration. The method does not depend upon cardiac maintenance of the circulation, adequate mixing of contrast media with circulating blood, or the use of expensive special radiographic equipment, nor is there any reasonable limitation to the degree of magnification attainable. It also lends itself readily to combined angiographic and histologic studies and to serial-time investigations.

GLOMERULONEPHRITIS: A CORRELATION OF HISTOPATHOLOGY IN NEEDLE BIOPSIES OF THE KIDNEY WITH THE CLINICAL COURSE. John S. Howe and (by invitation) Alvin E. Parrish, Veterans Administration Hospital, Washington, D.C.

The demonstration by Iversen and Brun in 1951, with subsequent confirmation by the authors in 1953, that needle biopsy of the kidney is a useful, feasible, and safe method for studying the histopathology of renal disease, offers a new and promising approach to the study of glomerulonephritis. Eleven cases of acute glomerulonephritis were studied clinically and by needle biopsy of the kidney. Six of the cases had more than one biopsy at various intervals up to 2 years. In addition, 10 cases of chronic glomerulonephritis were studied clinically and had needle biopsy of the kidney. The series includes cases which fall in the following groups: (1) Acute glomerulonephritis progressing to the subacute stage with death in uremia. (2) Acute glomerulonephritis with clinical recovery and varying degrees of resolution of the glomerular lesions. (3) Chronic glomerulonephritis in various stages.

Examples of each type were presented and the clinical-pathologic correlation discussed.

RENAL DISEASES AS STUDIED BY SERIAL KIDNEY BIOPSY. Conrad L. Pirani and (by invitation) Robert C. Muehrcke and Robert M. Kark, University of Illinois College of Medicine, Chicago, Ill.

During the past 18 months we have done over 200 needle biopsies of the kidney with the patient in the prone position. The procedure was usually painless, and representative samples were obtained in a high percentage of attempts. Neither serious hemorrhage nor dissemination of infection developed in the patients. The exact position of the kidney is located with a fine exploring needle, and the specimen is taken with the Franklin modification of the Vim-Silverman needle. A sandbag under the abdomen pushes the kidney against the back and provides hemostasis after biopsy. Biopsies have been taken from patients aged 9 to 67 years. In some of them as many as five biopsies were obtained at intervals varying from 2 weeks to several months.

Kidney biopsy is of value as it can either correct or confirm the clinical diagnosis. Treatment and prognosis can be placed on a more exact basis by the histologic findings. This technique also is useful in obtaining cultures of organisms from the kidney parenchyma and in assessing the effect of drugs on disease processes. In addition, serial biopsies are of obvious value in following the natural history of renal diseases including those which are reversible in character, in studying renal histology and histochemistry *in vivo*, and in obtaining data which may clarify present concepts of the physiology and pathophysiology of the kidney.

* * *

COARSE NODULAR CIRRHOSIS PRODUCED BY ETHIONINE. Hans Popper and (by invitation) Kevin Clearkin, Geoffrey Kent, Mabbu Parthasarathy, and Clara Bruce, Hektoen Institute for Medical Research of the Cook County Hospital, Chicago, Ill.

Rats kept on diets containing ethionine, the biologic antagonist to methionine, in excess of the methionine content of the dietary protein, within 4 weeks developed hepatitis with diffuse hepatocellular degeneration and regeneration, interstitial inflammation, ductular proliferation, and formation of intralobular connective tissue membranes. If the rats are kept on the same diet alternating with stock diets for prolonged periods, a coarse nodular cirrhosis develops in which the lobular pattern is dissected by thick trabecula containing proliferated ductules and inflammatory exudate. Some of the nodules consist of several preformed lobules; others, which

may reach 0.5 cm. in diameter, are composed of excessively regenerating liver cells, different from those in the surrounding liver tissue and not arranged in lobules. A third type of nodule which also may grow up to more than 1 cm. in diameter consists of proliferating bile ducts surrounded by connective tissue (cholangiofibrosis). In some instances such adenomatous structures contain large amounts of mucus, which also is free in the stroma. The cytologic appearance as well as the lack of sharp limitation of some of the larger nodules, raises suspicion of an adenocarcinoma, although the malignant character of the lesion is as yet not proved and metastases have not been found. Discontinuation of the ethionine diet for many months results in disappearance of the hepatocellular changes but not in restoration of lobular architecture; connective tissue septa still dissect the parenchyma. The cholangiofibrotic foci have undergone extensive fibrosis; however, some bile ducts still show bizarre proliferation with many mitotic figures. In some rats the changes in the coarse nodular cirrhosis simulate the picture found in rats on carcinogenic procedures shortly before unquestionable carcinoma appears.

The transition of the hepatitic stage into the cirrhotic stage was described. Functionally, the cirrhotic stage shows, in addition to the changes in hepatic enzymes found in the hepatitic stage and characteristic of hepatocellular damage, alterations of the serum proteins, specifically, marked elevation of gamma globulin which is not found in the hepatitic stage.

Injection Corrosion Studies of the Vascular Systems of Normal and Cirrhotic Livers. Milton R. Hales (by invitation), John S. Allan (by invitation), and Ernest M. Hall, University of Southern California School of Medicine and Los Angeles County Hospital, Los Angeles, Calif.

Livers from 61 patients necropsied at the Los Angeles County General Hospital were prepared as vinylite vascular corrosion casts. The series included 19 normal livers, 27 livers with Laennec's cirrhosis, and a total of 25 livers with other types of cirrhosis, tumors, or severe fatty change. In all cirrhotic livers, enlargement of the hepatic arteries and increase in the arterial bed were constant features. Arterial enlargement was most striking in vessels less than 1 mm. in diameter, using the size of the adjacent portal vein as the standard of reference; arteries equal in size or larger than the adjacent portal vein were noted in many severely fibrotic livers. Gross arteriovenous anastomoses were noted only within neoplasms, but unduly easy communication between arteries and portal veins was often apparent in Laennec's cirrhosis when a sufficiently dilute injection mass was employed. Equal and severe reduction and distortion were the general rule in hepatic and portal venous beds in the chronic atrophic stage of Laennec's cirrhosis. However, in many of the larger, severely fibrotic livers (subchronic phase), reduction and distortion in the hepatic venous bed were more severe than in the portal venous bed; some cirrhotic livers possessed an essentially normal gross portal venous bed, yet moderately or severely reduced and distorted hepatic venous bed. The major site of venous obstruction in cirrhosis may thus be on the hepatic rather than on the portal side of the venous system. The greatest disproportion between the hepatic and portal venous beds was noted in grossly visible areas of severe atrophy and fibrosis where there was little persistent or regenerated parenchyma. Atrophy and fibrosis would seem to be more significant factors in the obstruction of the hepatic veins than the expansile pressure of regenerating nodules. Small portal-hepatic venous anastomoses were demonstrated in several of the cirrhotic livers injected with a sufficiently limpid mass. They were also seen with severe focal or diffuse atrophy, and were extremely large and numerous in an example of so-called thyrotoxic cirrhosis.

KWASHIORKOR IN A WHITE AMERICAN MALE. Israel Diamond (by invitation), University of Louisville School of Medicine and Children's Hospital, Louisville, Ky.

An 11-year-old, red haired, white boy was admitted for study on several occasions for anasarca, dermatitis, and progressive depigmentation of the hair. The clinical and chemical features of his course were presented and the necropsy findings described. The observations correspond to those hitherto described only in African Negroes.

THREE-DIMENSIONAL ANALYSIS OF HEPATOCELLULAR CARCINOMA. Hans Elias (by invitation) and Hans Popper, Chicago Medical School and Hektoen Institute for Medical Research of the Cook County Hospital, Chicago, Ill.

Methods of three dimensional visualization, previously applied to the study of the normal and cirrhotic liver, have been extended to the study of hepatocellular carcinoma. This was done in order to clarify the morphogenesis of various structures in primary liver cancer. Preceding observations on the comparative embryology of the vertebrate liver served to find analogies between human liver cancer and forms of liver structure in embryonic and adult livers of various vertebrates. Reconstruction from serial sections revealed multiple transitions of one-cell thick plates (normal) into plates two cells thick, with the cells in the latter exhibiting the cytologic criteria of cancer. From this structure three different morphologic pathways can be traced representing the main descriptive types of hepatic cancer. (1) Globular masses of liver cells are noted without internal stroma surrounded by the original sinusoidal endothelium. (2) Plates two cells thick degenerate into flat "wallwork" which in the histologic sections resembles cholangioles. Their lumina do not contain bile. They are surrounded by excessive connective tissue. (3) Short tubules develop within the wallwork of plates two cells thick. The tubular lumina filled with bile are usually not connected with each other. It appears that hepatocellular carcinoma reproduces in reverse order phylogenetic and ontogenetic stages of the vertebrate liver.

HEMOPHILIC ARTHROPATHY IN DOGS. Margaret C. Swanton (by invitation) and K. M. Brinkhous, University of North Carolina, Chapel Hill, N.C.

Studies of canine hemophilia have been carried out on a colony of inbred dogs maintained in our laboratory for the past 7 years. Their clotting defect is identical with that of human hemophilia, and is similarly transmitted as a completely sex-linked recessive characteristic. The affected animals have been reared to maturity by frequent treatment with blood and plasma transfusions. Without treatment death usually occurs from massive hemorrhage in the first few months of life.

This report deals with the findings in the articular and periarticular tissues in these animals. The most common clinical manifestation of the disease in these dogs is recurring, painful, and occasionally swollen major joints in the extremities. At necropsy, following death from massive hemorrhage or intercurrent disease, the synovial membranes of large joints are the most frequent sites of fresh hemorrhage and of residua of previous hemorrhage. Evidence of recent bleeding consists chiefly of hemorrhage into the synovial membrane, with only about one third of joints thus involved showing blood within the joint cavity. The most common lesion is hemosiderosis of synovial tissue, with mild hyperplasia and usually without conclusive evidence as to whether blood has been present in the joint cavity. No lesions resembling "pigmented villonodular synovitis" have occurred. Damage to articular cartilages, adhesions between synovial surfaces, and capsular fibrosis sufficient to limit motion have been seen infrequently. The last appears more apt to occur when there also has been soft tissue hemorrhage outside the joint. The incidence of hemor-

rhage, both recent and old, in tendon sheaths and fasciae of the extremities is second only to that in synovial membranes of joints.

PATTERN OF ORGAN INFILTRATE IN ACUTE LEUKEMIA OF CHILDREN. Terence H. Cochran (by invitation) and Arthur Haut (by invitation), University of Utah College of Medicine, Salt Lake City, Utah.

The good therapeutic response of acute lymphoblastic leukemia to cortisone in contrast to the poor response of acute myeloblastic leukemia indicates the need for separation of acute leukemias into their various patterns. When the acute leukemias were classified on the basis of cytology as reviewed by one observer, it was found that there was an excellent correlation between this means of classification and their response to cortisone. Beard has shown a still further difference on the basis of the serum B_{12} level, which is elevated in acute myeloblastic, normal in acute lymphoblastic, and intermediate in monocytic leukemia.

In this study an attempt to differentiate the acute leukemias of children is made on the basis of pattern of organ infiltrate. Twenty-eight cases of acute leukemia (ages 0 to 11), necropsied during the 10-year period 1944–1954, are analysed. Twenty-one of these, on the basis of their histologic appearance, were classified as lymphoblastic and the remaining 7 as myeloblastic. A comparable number (20) of older children and adults with acute myeloblastic leukemia were studied also in order to evaluate further the difference in pattern of organ infiltrate. Of the 29 cases there was a difference of opinion in one, diagnosed by blood and marrow smears during life as acute myeloblastic leukemia and treated with aminopterin (5 mg. per day) for 7 days, but considered lymphoblastic on the basis of post-mortem morphology. The difference in pattern was apparent in spite of a wide variety of therapy. Four of the patients received no specific therapy for their leukemia, 16 received hormonal therapy with or without folic acid antagonists and/or 6-mercaptopurine, and 4 received x-ray therapy with or without nitrogen mustard, urethane, or folic acid antagonists.

MORTALITY IN RELATION TO HISTOLOGY IN HODGKIN'S DISEASE. Hans F. Smetana and (by invitation) Bernard M. Cohen, Armed Forces Institute of Pathology and National Research Council, Washington, D.C.

A follow-up study of Hodgkin's disease has been conducted on 437 cases in the Registry of Lymphatic Tumors of the Armed Forces Institute of Pathology. In every case the disease was diagnosed in white males in the Army during the period of World War II (1941–1946). Follow-up was complete and was accomplished through June 30, 1953, by means of service, Veterans Administration and other records of hospitalization, disability, and death. The histologic material was reviewed to confirm the diagnosis and to differentiate the histologic types: paragranuloma, granuloma, sclerosing granuloma, and Hodgkin's sarcoma. This first report of findings presents definitive life-tables for confirmed Hodgkin's disease by type, and evaluates the trend of survival both from apparent onset of symptoms and from diagnosis.

ON THE MECHANISM OF UNEXPECTED (SO-CALLED SPONTANEOUS) RUPTURE OF THE SPLEEN IN MALIGNANT LYMPHOMA. R. Philip Custer, Mary Jo Gunter (by invitation), Shields Warren, and Fred C. Collier, Presbyterian Hospital, Philadelphia, Pa., and New England Deaconess Hospital, Boston, Mass.

The infrequently occurring, yet teleologically warranted, unexpected splenic rupture in malignant lymphoma is associated with capsular changes of quantitative and qualitative nature rendering the organ maximally susceptible to minimal trauma. These manifest changes, to be reviewed in detail, suggest the pathogenesis of this

relatively rare, yet clinically dramatic event. The infrequency of unexpected splenic rupture in malignant lymphoma is attested by the fewer than 20 authenticated reports found in the literature.

Histologic and histochemical studies were effected on five ruptured spleens, each showing pathologic changes of one of the variants of malignant lymphoma, in an effort to find changes common to these spleens but not observed in so-called normal spleens, and not found in malignant lymphoma spleens which had not ruptured. Study of sections of 200 intact malignant lymphoma spleens has brought out that, "when the parenchyma is involved the capsule of the organ is virtually never spared." Mere metaplasia within the capsule was not sufficient of itself to set the stage for rupture, for this was evident in virtually all sections studied of ruptured and intact lymphoma spleens. It was therefore postulated that certain ancillary factors must be operative.

Remarkably trussed to resist external stresses and strains, the spleen is the only organ incased in a series of overlapping arches; the splenic arch formed by intervening capsule and adjacent trabeculae, serves to protect a turgid, fragile, relatively exposed organ. The integrity of the arch, violated by metaplastic cells, is further threatened by a chemical alteration possibly induced by these aberrant cells; stretching of the span and blunting of the leg-span angle is uniformly seen in splenomegaly. Of probably even greater import, however, is the structural alteration in collagen and elastica. Common to all ruptured spleens were demonstrable changes in the location of elastic tissue within the capsule, in direction, thickness, and tortuosity of elastic fibers, and more or less constantly in staining affinity of both collagen and elastic tissue. These changes were not observed in normal spleens forming the negative control group or in unruptured malignant lymphoma spleens, which served as a positive control group.

The structural and tinctorial differences existing between the capsules of the experimental group, and those of the negative and positive control groups of spleens suggested further investigation with the Polaroid Color Translating Ultraviolet Microscope. The absorptive behavior characteristics of splenic capsules of each of the groups were studied at 235, 240, 248 mμ; 248, 263, and 280 mμ; and 240, 263, and 280 mμ. Color photomicrographs of the results of these studies and of the histochemical studies were presented.

THYMOMA: A REVIEW AND RECLASSIFICATION. Lalla Iverson, Armed Forces Institute of Pathology, Washington, D.C.

True tumors of the thymus may be divided into two subgroups: those occurring in patients with symptoms of myasthenia gravis, and those not associated with this clinical syndrome. Examples of each of these two groups have a distinct histologic appearance. Both groups, however, are relatively benign in behavior. Tumors not associated with myasthenia gravis are represented by enlargement of the lymphoid or stromal elements of the gland in varying proportions. Depending on the type of tissue most prominent, various histologic classifications may be conceived. However, since similar reactions may be followed in thymus glands removed incidentally at necropsy, these changes in "non-functioning thymomas" are interpreted as representing essentially a hyperplasia or neoplasia of the entire gland (exclusive of its functional component) with one or more tissues being dominant at different stages —the lymphoid, the fibrous or reticulo-endothelial, or the angio-endothelial.

Thymomas associated with myasthenia gravis are characterized by a particular type-cell which is found in varying proportions and patterns in all examples of this series. When mixed with lymphocytes the resulting histologic picture is "lympho-epitheliomatous." If this type-cell happens to dominate the histologic field, the resulting impression is of an epithelial or glandular tumor. Because of the frequency

of its occurrence in thymomas removed from patients with myasthenia gravis, this type-cell is thought to be functionally related to the specific symptoms of this clinical syndrome.

Ten cases previously designated carcinoma of the thymus are now reclassified as tumors histologically and clinically corresponding to dysgerminoma of the ovary and seminoma of the testis. They are roughly grouped with teratomatous tumors (which are intrinsic to the mediastinum rather than to the thymus). These tumors in morphologic characteristics and in behavior are unlike true thymomas; and there is no conclusive embryologic or anatomical evidence to support theories relating to their thymic origin. The so-called "granulomatous form of thymic carcinoma" may be explained as a reticulo-endothelial reaction to the neoplastic cells of this tumor ("seminoma," "dysgerminoma," "germinoma").

In some series, mediastinal lymph node enlargement characterized by hyperplasia of the follicles and hyaline alteration of their germinal centers has been erroneously diagnosed as "thymoma." Five similar cases were included in this series for comparison with true thymomas.

INTRATHORACIC ANGIOMYOMATOUS HYPERPLASIA ASSOCIATED WITH CHRONIC CHYLOTHORAX. T. C. Laipply and (by invitation) J. C. Sherrick, Northwestern University and Chicago Wesley Memorial Hospital, Chicago, Ill.

Localized hyperplasia of smooth muscle in pulmonary bronchioles, vessels, and lymphatics has been observed frequently in chronic pulmonary disease. Hyperplasia of intrathoracic smooth muscle and vessels, sufficiently diffuse and striking to resemble tumor, has not been previously described in the English literature. In the foreign literature the condition has been observed to occur with tuberous sclerosis and with microcystic emphysema, with or without chylothorax. The condition has been referred to as angiomyomatosis of the lungs, muscular cirrhosis of the lungs, or honeycomb lung with myomatosis.

Two cases of chronic chylothorax are reported, in which there was striking proliferation of smooth muscle in lymphatics, blood vessels, bronchi, pulmonary interstitial tissue, mediastinal fibroadipose tissue, and mediastinal lymph nodes. In addition, there was a marked increase in vascular spaces. In some sites the hyperplastic smooth muscle and vascular channels formed tumor-like nodules. The condition is considered to be hyperplasia of lymphatics and smooth muscle (lymphangioleiomyomatous hyperplasia) resulting from chronic lymphatic obstruction. Lack of familiarity with this entity may lead to such erroneous diagnoses as benign tumor of mesenchymal tissue (angioleiomyoma, leiomyoma, fibroma), hamartoma, or even as metastatic tumor when present in lymph nodes.

HEALED PRIMARY COMPLEX IN HISTOPLASMOSIS. Manuel Straub (by invitation) and Jan Schwarz, University of Cincinnati College of Medicine and Jewish Hospital, Cincinnati, Ohio.

Healed histoplasmic pulmonary lesions were found in 65 per cent of 105 consecutive unselected necropsies in Cincinnati, Ohio. Organisms were demonstrated in the calcified pulmonary and/or lymph node lesions with the Gridley stain. The pathogenetic implications of these findings and the morphologic features of the primary complex were discussed.

PULMONARY MUCORMYCOSIS. Roger D. Baker and (by invitation) J. O. Wynn, Duke University School of Medicine and Veterans Administration Hospital, Durham, N.C.

A feature common to 5 fatal cases of mucormycosis of the lungs was the broad, branching, non-septate hyphae found in the lesions. The fungus penetrated the walls

of arteries and produced thrombosis. Veins and lymphatics were similarly involved, but less frequently. In one case, vascular thrombosis accounted for massive infarction of the lung, while other cases showed organisms widely spread in the bronchial tree and alveoli, producing pneumonia. Instances of involvement of hilar structures and diaphragm were noted. The lesions were acute, without organization, in 3 cases; subacute, with minimal organization, in a fourth case; and chronic, with giant cell and fibroblastic response, in a fifth. These represented durations of clinical symptoms of 5, 8, 10, 30, and 90 days. Two of the patients were 3 years old, and 3 were in the fifth decade. Cases were from Arizona, North Carolina (2 cases), South Carolina, and Texas. Evidence is advanced suggesting that Rhizopus may cause this form of mucormycosis. Diabetes was a predisposing condition in 3 of the cases, and acute leukemia treated by cortisone in one. In the remaining case no antecedent disease was known.

EXPERIMENTAL CEREBRAL MUCORMYCOSIS IN DIABETIC RABBITS. Heinz Bauer (by invitation), John F. Flanagan (by invitation), and Walter H. Sheldon, Emory University School of Medicine, Emory University, Ga.

Cerebral mucormycosis is an uncommon, generally fatal fungus infection occurring in patients with diabetic acidosis. It is clinically characterized by unilateral ophthalmoplegia and meningoencephalitis. This infection, until recently, has been diagnosed only at necropsy. The fungus had not been isolated, being classified as Mucor on its morphology in histologic preparations. We have performed necropsies on two diabetic patients with cerebral mucormycosis from one of whom the phycomycete *Rhizopus oryzae* was isolated. Extensive fungus invasion of the orbital tissues, meninges, brain, and of one ethmoid sinus was found.

One group of 9 rabbits with alloxan diabetes and 9 normal controls were inoculated intranasally with spores of *R. oryzae* suspended in saline solution. The diabetic animals were either sacrificed or necropsied 18 to 71 hours after inoculation. A control was sacrificed with each diabetic animal. Similar experiments were performed on three other groups of rabbits, each consisting of 3 diabetic animals and normal controls inoculated with other species of Mucorales isolated from patients with mucormycosis, i.e. *Rhizopus arrhizus, Absidia rammosi,* and *Absidia corymbifera.*

Marked fungus invasion of the nasal mucosa, blood vessels, and bone with extensive necrosis and acute inflammation was encountered in 7 diabetic rabbits of the first group. In four of these, meningo-encephalitis resembling cerebral mucormycosis in man was present. Renal fungus lesions occurred in 2 animals. One of the 2 rabbits without lesions had died 5 hours after inoculation while the other, sacrificed 168 hours after inoculation, failed to develop severe diabetes. All three diabetic rabbits inoculated with *R. arrhizus* showed nasal lesions with meningo-encephalitis in 2 and renal lesions in one. The 3 diabetic animals inoculated with *A. rammosi* displayed nasal lesions with meningo-encephalitis in one. Nasal lesions with meningo-encephalitis occurred once among the 3 diabetic rabbits inoculated with *A. corymbifera.* Fungus aspiration pneumonia was a frequent finding in all diabetic animals. Among the controls, few minute ulcerations of the nasal mucosa with acute inflammation and rare degenerating hyphae were seen in 5 animals of the first group and once in each of the other groups while cerebral, pulmonary, and renal lesions were lacking.

Our observations suggest that various species of Mucorales can produce cerebral mucormycosis with the paranasal sinuses representing the portal of entry. These fungi which are normally saphrophytic but become pathogenic in the diabetic host afford an opportunity for the study of the effects of altered metabolism on infection.

Read by Title

Cystic Disease of the Lungs: a Consequence of the Healing of Acute Disseminated Pulmonary Tuberculosis. Morgan Berthrong and (by invitation) Gustavo Ganem, Glockner-Penrose Hospital, Colorado Springs, Colo.

The widespread use of potent antibacterial drugs in human tuberculosis has led to certain quantitative changes in the pathologic features of that disease, although no fundamental alteration of tissue reactions in tuberculosis has been thus far described. One frequently noted observation has been the development of an extremely thin-walled cavity, probably resulting from rapid healing of a fresh cavitary lesion. Although such cavities were reported prior to the advent of antibiotics, thin-walled cyst-like lesions are seen with considerable frequency today. A necropsied case is described and 3 additional cases demonstrated by roentgenograms of generalized cystic disease developing after the improvement of acute disseminated pulmonary tuberculosis under treatment with antituberculosis drugs. At the necropsy of a 21-year-old Navajo Indian woman, who had been treated for 2 months with para-aminosalicylic acid, streptomycin, and isoniazid for what appeared to be a bilateral acute bronchogenic spread of tuberculosis, the lungs bilaterally contained innumerable smooth thin-walled cysts varying from several millimeters to several centimeters in size. The gross appearance was entirely compatible with congenital cystic disease. It was possible, however, to trace the development of these cysts from foci of caseous lobular pneumonia. Although the walls of the cysts were smooth and thin, microscopic epithelization had not occurred. An occasional epithelioid cell or focus of caseous débris was still present in the lining of most cysts and very rarely, tubercle bacilli. A more chronic fibrous cavity with caseous material was present in the right apex. The patient died of a perforated ulcer of the ileum. The development and persistence of multiple pulmonary cysts during the recovery from acute pulmonary tuberculosis presented a significant clinical problem in the other cases included in this report.

Systemic Effects of Carbon Particles Coated with Antigen. Uriel Garcia-Caceres (by invitation) and Bernard Black-Schaffer, University of Cincinnati College of Medicine, Cincinnati, Ohio.

Experiments were carried out to investigate the action of a localized antigen upon the vascular tree of a hypersensitized animal. The left cardiac ventricles of two groups of rabbits were inoculated with suspensions of carbon upon which bovine serum had been adsorbed for the experimental group, and, for the control group, homologous serum. All rabbits were injected intraperitoneally with 10 cc. per kg. of bovine serum. A second identical carbon particle series was given 5 days later and the animals killed at the end of a 12-day period. Two types of reaction were noted. One was a necrotizing granuloma which was attributed to the non-adsorbed antigen. It was seen in the peritoneum of both groups and the pericardium of the experimental group. The second reaction consisted of small intimal granulomas in prearteriolar vessels surrounding carbon particles. In the control animals there was no demonstrable reaction to the carbon coated with homologous serum.

Chlorophyllin Injection in Pregnant Mice. Susi Glaubach (by invitation) and William Antopol, Beth Israel Hospital, New York, N.Y.

It has been previously reported from our laboratory that chlorophyllin (sodium potassium copper chlorophyllin 75 to 85 per cent) administered subcutaneously to mice is taken up by all tissues except the brain and lung. When 20 mg. of chlorophyllin was injected subcutaneously in pregnant Paris mice, 5 to 8 days before

delivery and the mice killed at term, the uterus was pale green except at the site of placental attachment which was deep green. None of the fetuses showed any trace of green color in contrast to the placenta which was deep green. The placenta apparently acts as a barrier preventing chlorophyllin from entering the fetal circulation. Five chlorophyllin mothers delivered 32 young. No green color or any malformation was found in any of the offspring. The weight of the fetuses ranged from 1.2 to 1.4 gm., the same as that of the 38 delivered from 6 non-treated mothers. The milk suckled from the chlorophyllin mothers was white despite the deep green color of the mammary tissue. All 32 young developed normally, there was no retardation in body growth or in the initiation, rate, or density of hair growth as compared with the offspring from the control mothers. Thus the accumulation of chlorophyllin in the mammary glands does not interfere with the production of normally colored milk or with the nutritional composition of the milk as judged by the normal growth and development of sucklings from mothers treated with chlorophyllin 5 to 8 days before delivery.

In another group chlorophyllin was injected into pregnant mice 24 to 48 hours before delivery. The milk in the stomach of all sucklings had a green color after 6 hours. The milk was white again 48 hours later, despite the deep green color of the maternal mammary tissue. The disappearance of the chlorophyllin from the milk coincides with the disappearance of chlorophyllin from the urine and feces of the injected mouse, indicating that chlorophyllin is available for secretion only as long as circulating chlorophyllin is not fixed by the tissue. The sucklings showed no effects from ingesting the green milk.

APPLICATION OF THIN SECTIONS TO THE STUDY OF GLOMERULAR CHANGES IN GLOMERULONEPHRITIS. Edith Grishman (by invitation) and Jacob Churg, Mount Sinai Hospital, New York, N.Y.

Recent advances in microtomy, primarily in response to the needs of the electron microscopists, have presented a valuable tool also to those using the ordinary light microscope and the phase microscope. Only recently sections under 2 or 3 μ in thickness were rarely obtained. At present, serial sections measured in tenths or even in a few hundredths of a micron, can be produced with relative ease. Sections of the order of 0.5 μ greatly facilitate the study of normal architecture and its alteration in disease of complex structures such as the renal glomerulus. In view of rapid advances of the electron microscopy, any study with the light microscope can no longer be considered adequate. However, for the histopathologist ultra-thin sections provide a valuable intermediate step from the conventional preparations to the electron microscopy with its high magnification, small fields, and lack of color.

Diseases of the glomerulus can be best described in terms of alteration of its four component spaces: the epithelium-lined (Bowman's), the endothelium-lined (intracapillary), and the two mesenchymal spaces—pericapillary (potential) and intercapillary. The relationship of these components is roughly analogous to that of the peritoneal cavity, the lumen, and the wall of the small intestine and the mesentery. In the study of hyaline deposits in the glomeruli (Churg and Grishman) it has been shown that such deposits occur predominantly in the mesenchymal spaces, in the manner characteristic of each of the several diseases.

In glomerulonephritis, all four spaces participate to a varying extent, depending upon the stage and form of the disease. The most pronounced changes of the acute and the subacute stages were observed in the endothelium-lined and the epithelium-lined spaces, less prominent changes in the mesenchymal spaces. The proliferated endothelial cells in the capillary lumina are often outlined by thin PAS-positive fiber-like structures, which may represent attempts at formation of basement mem-

branes. Similarly, fibers in the epithelial crescents often appear to arise from the capsular basement membrane and probably represent newly formed epithelial basement membranes. Edema, exudation of inflammatory cells, and proliferation of mesenchymal cells are seen in the intercapillary space. Similarly, edema can be observed in the pericapillary space, particularly in cases associated with a clinical nephrotic syndrome. This edema leads to thickening of the capillary wall. The main finding in chronic glomerulonephritis is obliteration of the capillary lumina by collapse of the capillary walls. This is caused partly by compression of the capillary tufts by epithelial and later hyaline crescents in the Bowman's space. We have not encountered clear evidence of fibrosis of the capillary lumina, though such fibrosis affecting only a short segment of each capillary would be sufficient to collapse the whole capillary loop. Fibrosis undoubtedly occurs in the intercapillary space.

SUSCEPTIBILITY OF ENDOCARDIUM OF ALTITUDE RATS TO VARIOUS BACTERIA. Benjamin Highman and (by invitation) Paul D. Altland, National Institute of Arthritis and Metabolic Diseases, Bethesda, Md.

Bacterial endocarditis is readily induced in altitude rats (exposed daily to simulated high altitude) by intravenous injections of streptococci of viridans type. To determine their susceptibility to other bacteria occasionally causing human endocarditis, paired groups of altitude rats and ground level controls, 6 to 12 per group, received intravenously 0.5 cc. of young broth cultures of various bacteria. Rats were killed usually 6 days after the first inoculation. Microscopic studies revealed no unequivocal valvular lesions in either ground level or altitude groups given 1 to 7 inoculations of *Escherichia coli* K12, 3 of *Corynebacterium pseudodiphtheriae* 302, 4 of *Neisseria catarrhalis* N-6 (2076), 4 of alpha hemolytic Streptococcus (Lancefield M), or 1 or 2 of *Salmonella oregon* 909. Rats died within 48 hours after an inoculation of *Pseudomonas aeruginosa* (group 1), but showed no unequivocal valvular lesions. Severe endocarditis occurred in 5 of 8 ground level controls and in 7 of 8 altitude rats dying or killed within 72 hours after the first of 2 successive daily injections of type I Pneumococcus. Severe endocarditis occurred in 10 of 13 altitude rats given 1 or 2 inoculations of *Staphylococcus albus*, and in only 2 of 14 controls. Significantly, following 3 inoculations of *Hemophilus parainfluenzae* 655, severe endocarditis was noted in 8 of 16 altitude rats, but in none of 17 ground level controls. These findings indicate that altitude rats may be useful in studying *in vivo* the therapy and pathogenesis of endocarditis due to certain strains of Hemophilus, Staphylococcus, and Pneumococcus.

CORRELATION OF GLOMERULAR FILTRATION RATE WITH HISTOPATHOLOGIC CHANGES IN GLOMERULI AS SEEN IN NEEDLE BIOPSY OF THE KIDNEY. John S. Howe and (by invitation) Alvin E. Parrish, Veterans Administration Hospital, Washington, D.C.

The glomerular filtration rate (GFR) was determined by the inulin clearance method in 33 patients, including patients with various forms of renal disease and patients without renal disease. Needle biopsy of the kidney was performed in each case within 3 days of the GFR determination. Abnormalities of the GFR were graded as 0 to 4 plus impairment. Histopathologic changes in the glomeruli were graded 0 to 4 plus. A fairly good correlation was observed between GFR impairment and glomerular changes. In general, markedly decreased GFR was found only in cases in which glomerular changes were both severe and diffuse; slight to moderate impairment of GFR was associated regularly with slight to moderate glomerular changes, particularly, slight to moderate thickening of the glomerular

basement membranes. Examples are shown of the types of histopathologic lesions of glomeruli associated with slight, moderate, and severe impairment of the GFR.

LESIONS OF THE INTRAHEPATIC PORTAL RADICLES IN MANSON'S SCHISTOSOMIASIS.* Francisco Lichtenberg (by invitation), University of Puerto Rico School of Medicine, San Juan, P.R.

With the exception of pulmonary arteritis, the vascular lesions of Manson's schistosomiasis have received little attention. However, there are a few descriptions of vascular inflammation in the colon and liver, including a massively infested case with penetration of eggs into the greater circulation, presumably through arteriovenous pulmonary anastomoses. The impacted ova were in arteries and were surrounded by miliary granulomas. A systematic search for intrahepatic vascular lesions was therefore undertaken and by employing special stains and multiple sections, such lesions were detected in 19 of 27 cases studied (70.4 per cent).

The lesions of the portal radicles were: (a) *Substitution by granuloma* so that the radicle is replaced and obstructed by a pseudo-tubercle surrounding a parasitic egg. Occasionally, remains of elastic fibrils can be demonstrated. Successive steps in the development of this lesion were described, beginning with an endothelial proliferation, giant cell formation, and inflammatory infiltration of the portal space. This lesion was observed in early cases and is not correlated with signs of portal hypertension. (b) *Fibrosis with narrowing* is found in the more advanced cases, often together with portal hypertension and always in conjunction with fibrosis of the portal spaces. This lesion probably represents a residual stage of a previous inflammatory process. (c) *Intrahepatic thrombophlebitis* of the portal radicles is the severest lesion encountered. It occurs only in advanced cases which also show pipe-stem cirrhosis, and it is always accompanied by portal hypertension. There is an organizing and inflamed thrombus; all three layers of the vein are infiltrated by inflammatory cells; eggs and granulomas can be seen in the thrombus and in the vascular wall, which is disorganized and shows interruptions in the continuity of elastic membranes and fibrils. This lesion may be thought of as analogous to the arteritis occurring in severe infestation of the lungs and, as in the latter, numerous dilated capillaries with an angiomatoid pattern may be present.

Since intrahepatic vascular lesions arise early in Manson's schistosomiasis and since they are frequently severe in the advanced cases, they may offer a clue to the cause of the predominance of portal hypertension in clinical cases.

FURTHER STUDIES IN QUANTITATIVE CYTOPATHOLOGY: OPTICAL MEASUREMENTS OF THE ORGANIC MASS AND THE NUCLEOPROTEIN MASS OF CHROMOSOMES IN NORMAL AND NEOPLASTIC CELLS. Robert C. Mellors and (by invitation) Louis G. Ortega, Memorial Center, New York, N.Y.

The interferometric method for optically measuring the total organic mass (dry weight) of minute cell structures *in situ*, such as chromosomes, has been described recently. It is now possible after suitable fixation, lipid extraction, dehydration, and mounting of cytologic materials, to determine the quantity (content) of nucleoprotein in chromosomes. The content of nucleoprotein is found to be different in the sperm nuclei of certain species of animals; to be half as great in these sperm nuclei as in a double set of germinal chromosomes; to be equally distributed between the sets of chromosomes in daughter cells after normal cell division; to be the same in prophase and metaphase sets of chromosomes; to be characteristic of chromosomes within a set; and to be very much increased in metaphase sets of chromosomes in certain types of cancer cells of the mouse (sarcoma 180, Ehrlich and Krebs-2 ascites tumors), thus reflecting an increase in the number or the size of the chromosomes.

* This article will appear in a subsequent issue of *The American Journal of Pathology*.

RESISTANCE TO QUADRUPLE COMBINATION CHEMOTHERAPY IN LEUKEMIA L1210 IN MICE. E. M. Nadel and (by invitation) A. G. Hilgar, National Cancer Institute, Bethesda, Md.

A better understanding of the mode of action of drugs effective in the chemotherapy of leukemia L1210 in mice has been sought in studies on synergism using the principle of combinations of drugs at dose levels where each is individually ineffective. In further studies, amethopterin at 1.5 mg. per kg., or 2,4-diamino-5(3′,4′-dichlorophenyl)-6 methyl pyrimidine (BW-50-197) at 1.25 mg. per kg., or 2,6 diaminopurine at 47 mg. per kg., or ethionine at 75 mg. per kg. were individually administered as the sole therapeutic agent (intraperitoneally every other day, beginning 3 days after subcutaneous transplantation). Each alone was found to be ineffective in controlling leukemia L1210; survival time, spleen size, and local tumor growth were not significantly different from those of untreated controls. In seven series (238 mice) in which the four drugs were administered in combination at the individually ineffective doses, survival was 18.3 ± 1.1 days compared to 8.6 ± 0.25 days for untreated controls; and there was a significant increase in survival time over that observed after all other combinations of these drugs at these doses. With repeated passage (10 mice per passage) stable resistance to these four drugs has developed in two sublines of leukemia L1210. Two resistant lines, now in their 28th transplant generation, are being utilized in studies on the mode of action of drugs structurally related and unrelated to folic acid, purine, pyrimidine, and methionine analogues.

HUMAN LUNG IN LEUKEMIA: OBSERVATIONS ON ALVEOLAR CAPILLARY LEUKOSTASIS WITH REFERENCE TO PATHOLOGIC PHYSIOLOGY. Jacob W. Old, Wirt W. Smith, and Giuseppe Grampa (all by invitation), University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas.

We have been able to find only a few references to specific changes in the lungs, with leukemia, none of which emphasize pulmonary leukostasis. We have encountered an overwhelming engorgement of pulmonary alveolar capillaries in 8 of 16 cases of leukemia, which is strikingly characteristic. The lungs grossly are voluminous and almost completely aerated. They have a pallid yellowish color. The cut surfaces are peculiarly dry, and generally neither edema fluid nor blood can be expressed. The microscopic sections show almost complete distention of all alveolar capillary spaces by engorgement with leukemic cells, and the alveolar spaces remain rigidly expanded without evident fluid content.

These cases were re-examined as a group with emphasis on clinicopathologic correlation. It was found that changes as described were associated with enormously elevated leukocyte counts in the peripheral blood (350,000 to 600,000 per cmm.), and that the most usual leukemic type was the chronic granulocytic process. Of the 9 cases of definite granulocytic leukemia which are included in this series, the 5 cases which showed elevated counts also showed capillary engorgement, while the 4 cases with low counts showed no essential pathologic change. Of 3 cases of monocytic leukemia, 2 showed elevated leukocyte counts associated with alveolar capillary engorgement. The remaining 4 cases were of the lymphocytic type and only one of these showed an elevated count and alveolar capillary engorgement in a case in which the definite diagnosis of the leukemic type was in some doubt.

The pulmonary circulation operates at about one fifth of the pressure present in the systemic circulation, and depends in part on static gravitational forces and thoracic pressure changes for proper function. We feel that the sequestration of leukemic cells in the pulmonary capillaries is partially the result of low hemodynamic pressures which tend to cause stasis of the relatively large leukemic cells in the physiologically stagnant capillary system of the lungs.

RELATION OF SPLENIC CALCIFICATION TO HISTOPLASMOSIS. Jan Schwarz, Frederic N. Silverman (by invitation), Manuel Straub (by invitation), Seymour Levine, and Salvador Adriano (by invitation), University of Cincinnati College of Medicine, Jewish Hospital, and Children's Hospital and Children's Research Foundation, Cincinnati, Ohio.

X-ray examination of spleens in the endemic area of histoplasmosis reveals a much higher incidence of calcifications than comparative series from Holland and New York. The morphology of the lesions is described, the organisms demonstrated by Gridley stain, and the pathogenesis of the splenic lesions discussed.

BASOPHILIC DEGENERATION OF THE MYOCARDIUM. Thomas M. Scotti, University of Miami School of Medicine, Coral Gables, Fla., and Jackson Memorial Hospital, Miami, Fla.

A histologic examination of the hearts in 75 consecutive necropsies was made in order to study basophilic degeneration of the myocardium. Seven areas of each heart were examined: both ventricles, the atria, the atrial appendages, and the interventricular septum. Each section was stained by hematoxylin-eosin and periodic acid-Schiff (PAS) techniques. In the group of cases with basophilic degeneration, representative sections were stained by the following methods: PAS with digestion, toluidine blue, Mayer's mucicarmine, Feulgen, crystal violet for amyloid, Turnbull's blue for iron, von Kossa, and Giemsa. Also some sections were treated with ribonuclease and hyaluronidase.

The basophilic character of the lesions was well seen in the hematoxylin-eosin sections; but in some instances the lesions were very pale and not distinct, in which state they were interpreted as the early phase of basophilic degeneration. The areas of degeneration stained brilliantly with PAS and remained just as brilliant after digestion with diastase. Metachromasia was observed with toluidine blue. Mucicarmine stained the material light red. The Feulgen reaction and ribonuclease test were negative and the other stains did not impart any specific color to the material. The studies with hyaluronidase suggested that the basophilic substance, which was found to be of a mucinous nature, was hyaluronidase-fast. In a large number of the sections with this form of degeneration, lipochrome pigment was very prominent. The relationship of this pigment to the basophilic substance was not determined. Basophilic degeneration was found most frequently in the left ventricle and then in the interventricular septum. The degree of change was recorded as "slight" or "considerable." It was regarded as "slight" when only occasional fibers were affected in the entire section. Sometimes only a single fiber with basophilic degeneration was demonstrated. The degree of degeneration was regarded as "considerable" when more fibers were affected, usually several fibers in many areas of the section. There was no predilection for any particular level of the myocardium.

Of the 75 cases, there were 53 (70.6 per cent) with basophilic degeneration. In 41 of these, at least two or more areas of the myocardium were affected. The degree of basophilic change in 37 cases (49.3 per cent) was "slight" in all sections. Among this group there were 11 cases with only early lesions. In 16 cases (21.3 per cent) the degree of change was "considerable" in at least some sections. The over-all incidence of this form of degeneration was higher than anticipated. This was probably due to the examination of multiple sections of each heart, and to the routine use of the PAS method. The latter permitted the ready identification of the early phase which otherwise might have been overlooked in the corresponding hematoxylin-eosin section.

Basophilic degeneration was found usually in patients above the age of 40 years; but that it could occur earlier was evidenced by the fact that 3 of the patients were 11, 13, and 16 years of age. There was only a slight predominance in men over women

affected. Myxedema did not exist in any of the patients. The degeneration was not attributed to post-mortem change. Upon reviewing the causes of death or the main pathologic findings observed at necropsy, it was not possible to consider any particular disease as the factor in producing basophilic degeneration of the myocardium; but the possible relationship to other cardiac disease was discussed. The cardiac lesions observed in the adult group were coronary atherosclerosis, myocardial infarcts, focal myocardial fibrosis, and cardiac hypertrophy. All 3 young patients had cardiac hypertrophy. In one, it was due to chronic rheumatic carditis with acute exacerbation; in the second, hypertension was associated with chronic glomerulonephritis; and in the third the cause was undetermined.

EFFECT OF AUDIOGENIC STIMULI ON WOUND HEALING IN DBA/1 MICE. David M. Spain and (by invitation) Norman Molomut and Bernard Riess, Beth-El Hospital, Brooklyn, N.Y., and Waldemar Medical Research Foundation, Port Washington, N.Y.

In order to evaluate the effects of a specific form of stress as opposed to the gross and nonspecific procedures used in most experimental studies, the effect of subconvulsive exposures to audiogenic stimuli in a susceptible dba/1 strain of mice on wound healing was studied. Dba/1 mice between the ages of 5 to 10 weeks from the same litters were exposed to bell ringing in a metal washtub under standardized conditions for periods ranging from 30 to 90 seconds daily. The exposure was just short of that necessary to produce convulsions. The pre-convulsive manifestations consist of marked racing and jumping. After the first exposure to audiogenic stimuli uniform wounds were made on the back of each mouse. Control groups were wounded and cared for in a similar manner except that they were not exposed to bell ringing. At the end of the sixth day post-wounding in the control animals all the wounds showed considerable healing. Most of these were completely epithelized. In the group exposed to audiogenic stimuli, very little healing of the wounds was observed and microscopically there was only sparse granulation tissue formation with slight epithelization. Whether this mechanism of suppression of wound healing by a specific form of neurogenic stress is mediated through the pituitary-adrenal axis is under investigation.